Cannabinoids and Psychosis – Cause and Treatment

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PREFACE

The author uses boxes and and appendices in the hope that the main text flows better. Where a particular topic requires more space, the reader will be directed to a note in appendix 2.

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ABSTRACT

Epidemiological studies suggest that cannabis is a risk factor for psychotic illness. The main active ingredient is Δ-9-Tetrahydrocannabinol (THC). In healthy humans, the acute administration of THC can elicit transient psychotic symptoms and cognitive impairment. THC stimulates the endocannabinoid CB_1 receptor (CB_1R). However, beyond CB_1Rs, the mechanism underlying the pro-psychotic effects of THC is unknown. *The exploration of candidate mechanisms was the first major theme in this thesis.* In Study 1 the pro-psychotic properties of intravenous (IV) THC were confirmed. Thereafter, studies 2 and 3 explored whether the pro-psychotic effects were related to excess striatal dopamine release or abnormal neural oscillations respectively.

The cannabis plant contains over sixty cannabinoid molecules, one of which, Cannabidiol (CBD) can antagonise some of the pharmacological effects of THC. It has been suggested that the absence of CBD in modern, 'high-potency' forms of cannabis (sinsemilla or 'skunk') underlies the risk of such preparations for mental health. However the evidence for this is sparse. *Characterising the effect of CBD on THC-elicited responses was the second major theme in this thesis*. Studies 4 and 5 tested whether CBD inhibited acute THC elicited psychosis.

In study 1 the psychotomimetic effects of acute IV THC were confirmed. THC-elicited positive symptoms were distinct from anxiety, and negative symptoms were distinct from sedation. Cognitive performance was impaired under THC conditions. In study 2, THC had no significant effect on striatal dopamine release despite inducing robust positive psychotic symptoms. In study 3, THC-elicited positive psychotic

symptoms were related to reduced bi-frontal coherence in the theta (4-8Hz) band. Studies 4 and 5 both showed that CBD pre-treatment inhibits acute THC-elicited psychosis.

Overall two major findings emerged. 1. The pro-psychotic effects of THC were related to abnormal neural oscillations, but not to striatal dopamine release; and 2. Cannabidiol inhibits acute THC psychosis.

INTRODUCTION

Psychosis

In general psychosis refers to the presence of hallucinations (false perceptions), delusions (false, fixed ideas, which carry overwhelming significance for the patient) and thought disorder. For over 100 years the psychoses have been divided into organic and functional categories.

Organic denotes an identifiable systemic or central pathology. Organic psychoses can be secondary to endocrine disorders (*thyroid disease*); metabolic disease (*acute intermittent porphyria*); autoimmune disorders (*paraneoplastic limbic encephalitis*); infection (*herpes simplex encephalitis*); seizures (*temporal lobe epilepsy)*; space-occupying lesions; stroke; head-injury; demyelinating diseases (*metachromatic leukodystrophy*); neurodegenerative disease (*Lewy-body dementia*); basal ganglia disorders (*Wilson's disease*); nutritional deficiencies (B12 deficiency); medications (*acyclovir*); environmental toxins (*thallium*); and psychoactive drugs (LSD).

The identification of an organic psychosis depends upon a thorough history, physical examination and the prudent use of laboratory investigations. Identification of an organic cause of the psychosis can dramatically change the subsequent management and prognosis.

Functional psychoses are diagnoses of exclusion (i.e. exclusion of identifiable organic pathology). There are as yet no diagnostic tests. Diagnosis is made of clinical grounds (symptoms/signs) according to the criteria in the Diagnostic & Statistical Manual of the American Psychiatric Association (APA, DSM-IV-TR) or the International

Classification of Diseases of the World Health Organisation (WHO, ICD-10). The two classification systems are broadly similar. They subdivide the functional psychoses into schizophrenia (*paranoid type, disorganised/hebephrenic type, catatonic, undifferentiated, residual* [*and simple in ICD-10*]); persistent delusional disorders, schizophreniform disorder (DSM-IV-TR), brief psychotic disorders and schizoaffective disorder. Psychotic symptoms can also occur in bipolar disorder and major depressive disorder.

For a DSM-IV-TR diagnosis of schizophrenia, the following criteria must be met: 1. The presence of characteristic symptoms [at least two, (or one if delusions are bizarre/ or if auditory hallucinations form a running commentary or discuss the patient.)] for most of the time for one month (or less if treated), which can be delusions, hallucinations, disorganised speech, grossly disorganised behaviour or negative symptoms (*blunted affect, alogia or avolition*). 2. Social or occupational dysfunction. 3. Continuous signs of disturbance for six months (including one month of psychotic symptoms). Caveats are that the symptoms cannot be secondary to a mood disorder, a pervasive developmental disorder, or as a result of an identifiable organic illness.

Schizophrenia has a global lifetime prevalence of 0.3-0.7%[1]. Family, twin and adoption studies have shown that the vulnerability for schizophrenia is partly genetic, with heritability estimated at approx. 80%, but the molecular genetics of schizophrenia remains unresolved[1]. A small proportion of cases can be explained by rare copy number variations (CNV's)[2]. In addition numerous risk alleles have been proposed, the evidence being strongest for disrupted in schizophrenia-1 (DISC1) and neuregulin-1 (NRG1)[3, 4]. That many of the candidate genes are associated with other

neuropsychiatric syndromes, such as epilepsy and autism, serves to highlight the deficiencies in our current systems of classification[5]. Environmental factors which have been shown to confer risk for schizophrenia are; early life adversity, growing up in an urban environment, minority group position and cannabis use[6]. An influential view, which has stimulated much research, is that schizophrenia is a neurodevelopmental disorder, which arises from the interaction between risk genes and environmental risk factors[1].

In schizophrenia the most effective intervention is treatment with dopamine D2 receptor antagonist drugs[1]. Such drugs ("*neuroleptics*", "*anti-psychotics*") are particularly helpful for hallucinations, delusions and agitation – although they do little for the negative symptoms. The efficacy of the first drug, chlorpromazine was discovered by chance in 1951-52. The efficacy of haloperidol was discovered by design [see below] in 1958. Numerous attempts have been made to discover drugs based on alternative mechanisms, in an attempt to avoid the Parkinsonian side effects of the D2 antagonists and to extend efficacy to the negative and cognitive symptoms[7]. Newer agents such as olanzapine and quetiapine ("*atypical anti-psychotics*", "*second generation anti-psychotics, SGAs*") are largely devoid of Parkonsonian effects, but their efficacy is still based on D2 antagonism[1].

Of the 'atypicals' only clozapine has demonstrable efficacy against negative symptoms, but the basis for this remains unknown, and side-effects limit it's widespread use[8]. Although atypicals such as risperidone and olanzapine have become the first-line treatment, recent studies have cast doubt on whether they offer a significant advantage over the older drugs[9]. Instead of motor side effects, the

clinician's concern is that the atypicals are associated with obesity, dyslipidaemia and type II diabetes, increasing the risk for cardiovascular disease.

Drug Models of Psychosis

Several drug classes are said to be psychoto-mimetic. These are the stimulants (amphetamine/cocaine), the psychedelics (LSD), the NMDA-channel blockers (ketamine) and the cannabinoids (THC), amongst others. At one time or another, representatives of each class have been advanced as 'drug-models' of endogenous psychotic illness[10].

Whatever the substance, the following argument has been used to support the usefulness of the drug-model psychosis: *Since on the surface, the psychological manifestations of the drug induced psychosis and the illness are sufficiently similar, then their organic bases might also be akin. Thus, knowing about the mechanisms of the drug may inform about the, hitherto unknown, organic basis of endogenous mental illness.* Drug-models have also been prominent in the discovery of medications for psychosis/schizophrenia[11].

This approach has been remarkably successful in the case of the amphetamine/cocaine model; the model itself becoming one of the main pillars of the Dopamine Hypothesis – now in its 3rd version, and arguably as 'dominant' within psychiatry as at any other time in the past half-century. A high-point of the *dopamine-school* was the recognition of schizophrenia–like psychoses in professional cyclists who abused amphetamine, and the realisation that a drug which blocked the effects of amphetamine in animals could be beneficial for endogenous psychotic illness[12]. This

reasoning led to the discovery of haloperidol (subsequently identified as a potent dopamine D2 receptor antagonist), which swiftly translated to the psychiatric clinic. Haloperidol, and medications like it, completely changed the landscape of psychiatric practice[12].

With this success, a similar endeavour was attempted with the LSD-model psychosis. Drugs which block the effects of LSD in animals were quickly discovered (latterly shown to be serotonin $5HT_2$ antagonists), however such compounds turned out to be ineffective against the core symptoms of schizophrenia[10]. This gave weight to the well-known argument that the effects of LSD were, in any case, quite unlike endogenous psychosis, the former being characterised by visual experiences, the latter by pathological experiences in the auditory domain[10].

The origins of the ketamine-model and *the glutamate-school* go back to the 1960s. It was realised that ketamine (and the related molecule, phencyclidine) could elicit profound changes in thinking and behaviour[13]. Such molecules (shown in the 1980's to be NMDA channel blockers) were said to mimic not only the positive symptoms of schizophrenia, but the negative symptoms as well, and 'explanations' of endogenous psychosis based on glutamate emerged as a rival to the dopamine hypothesis. As was the case for dopamine, there has been considerable effort to identify *schizophrenia-related* pathology in the glutamate system, usually focussed on receptor numbers or concentrations of glutamate in the brain[14]. There has also been a hope that a glutamate drug would prove beneficial against the core symptoms of schizophrenia, translate to the clinic, and in doing so - validate the model. Unfortunately, and similar to the case with the serotonin-model, this has not yet occurred [but see Patil et al 2007 Nat Med 13:1102-7][15].

The Cannabinoid Model Psychosis

The final class to consider, and the subject of this thesis, is the cannabinoid-model psychosis. From the perspective of the current day, cannabis is the prototypical drug-model psychosis. For example, the world's first known pharmacopoeia, the Pên-ts'ao (The Herbal), compiled in the first century AD, documents, rather succinctly; *"Medical cannabis - stop eating. Eat more - you will see white ghosts. And eat long-enough - you will know how to talk with the gods*" (1234AD edition).

In 1840s Paris, many of the leading painters and writers of the day met as the *Club de Hashischins* and ingested what were described as large quantities of the drug. Their founding member, Jacques-Joseph Moreau Du Tours (1804-1884), a psychiatrist from the Bicêtre considered that "*Hashish gives to whoever submits to its influence the power to study in himself the mental disorders that characterise insanity, or at least the intellectual modifications that are the beginning of all forms of mental illness".*
The insight that a drug-model could be useful for exploring endogenous mental illness probably originates with Moreau Du Tours. His awareness of the links between "intellectual modifications" and mental illness was especially prescient, and such links are now commonplace in modern psychiatric thinking[16].

From the 1950's until the 1970's, there were occasional reports confirming that acute THC and cannabis could elicit transient psychotic-like phenomena[17-21]. Interest in central cannabinoid pharmacology was marginal however, until two streams of knowledge began to attract attention within their respective fields. In neuroscience it was becoming clear that the recently discovered endocannabinoids were transmitter-like signalling molecules (which functioned as mediators of specific forms of synaptic

plasticity)[22-26]. In psychiatry there were more and more reports of a link between cannabis-use and schizophrenia[27, 28].

Endocannabinoid Transmission

Cannabinoid Receptors

Modern cannabinoid research stems from elucidation of the structure of THC by Raphael Mechoulam and colleagues in the 1960s[29]. THC elicits psychological effects via partial agonism at the CB1 receptor, first identified in 1988[30] and cloned in 1990[31]. The CB2 receptor was initially discovered in macrophages[23] and was initially designated as the "peripheral cannabinoid receptor" or the "immune cell cannabinoid receptor"[32]. However recent evidence indicates that the CB2 receptor is expressed on activated microglia in the CNS, and probably on some neurons[32, 33].

Endogenous Ligands

As for opiate research two decades earlier, the discovery of cannabis receptors prompted a search for endogenous agonists and surprisingly the endocannabinoids (eCBs) turned out to be lipids. The first eCB, N-arachidonoylethanolamide (AEA) was termed Anandamide, from the Sanskrit word *Ananda*, signifying "bliss"[24]. A second eCB, 2-arachidonoylglycerol (2-AG) was discovered in 1995[25, 34], and others followed. To date AEA and 2-AG have been studied most intensively. Other putative endocannabinoids include 2-arachidonyl glyceryl ether (noladin ether)[35] and O-arachidonylethanolamine (virodhamine)[36]. Unlike conventional neurotransmitters/modulators, eCBs are not stored in vesicles, but synthesized 'on

demand' from membrane phospholipids[37, 38].

Retrograde Signals

In 2001, an eCB was identified as the long-sought retrograde mediator underlying the electrophysiological phenomenon known as depolarising induced suppression of inhibition (DSI)[22]. Endocannabinoids act as retrograde signals at CNS synapses[37-39]. They are synthesized in dendrites but act presynaptically to inhibit the release of fast-acting amino-acid neurotransmitters. (Thus 'neurochemical information' passes in a retrograde direction; from dendritic spines → axon terminal). Ultrastructural analyses have located key enzymes for eCB synthesis at dendritic spines, and have detected CB_1 receptors on the terminals of neighbouring GABA- and glutamatergic neurons[40-42]. In the CNS the CB_1 receptor is a coupled to $G_{i/o}$-type G-proteins, which mediate inhibitory effects at pre-synaptic terminals. The short-term suppression of transmitter release is via inhibition of voltage-gated Ca^{2+} channels[43] and activation of K^+ channels whereas long-term inhibition depends upon 2nd messengers (inhibition of adenylate cyclase) and inhibition of the vesicular fusion process[44, 45].

In the neo-cortex, striatum and hippocampus, CB_1R expression is considerably higher on GABAergic than glutamatergic terminals[40, 46-48]. The reason for this, and whether this pattern is seen throughout the CNS, remains unknown. Endocannabinoids are synthesized by principal output neurons such as Purkinje cells in the cerebellum, pyramidal neurons in the hippocampus and cortex, medium spiny neurons in the striatum, and dopaminergic neurons in the midbrain[49]. By releasing endocannabinoids, principal neurons are able to regulate their excitatory and inhibitory inputs. Retrograde endocannabinoid transmission constitutes another layer of complexity in the modulation of synaptic strength.

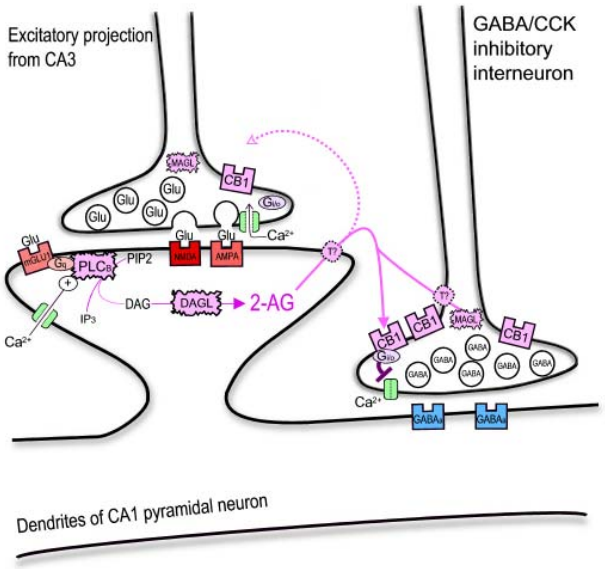
Synaptic Plasticity

Endocannabinoids have emerged as essential mediators of several forms of transient (5-30s) and long-term (>1 hr) plasticity in the cortex, limbic system, basal ganglia and cerebellum[22, 26, 50-53]. So far in all cases, eCB-dependent plasticity is expressed pre-synaptically as a decreased probability of neurotransmitter release. At the behavioural level, intact eCB signalling is required for cerebellar-dependent motor learning[54] and for extinction of aversive memories in the amygdala[55]. At CA3-CA1 synapses in the hippocampus, eCBs appear to facilitate memory encoding. Activity-driven synthesis of 2-AG in dendritic spines of pyramidal neurons leads to long-term depression of neighbouring GABA and cholecystokinin (CCK) terminals, so that adjacent excitatory synapses are primed for strengthening by reducing their threshold for long-term potentiation (LTP)[56] (Box 1A). Endocannabinoids also mediate a novel form of plasticity called, spike-timing-dependent-LTD[26].

Recent research indicates that endocannabinoid-mediated synaptic plasticity is likely to employ 2-AG as the retrograde signal[57]. The synthesis of 2-AG is driven by the stimulation of type I metabotropic glutamate receptors and Ca^{2+} entry via voltage operated channels, which activate the synthetic enzyme sn-1 diacylglycerol lipase (DAGL-α) (Box 1A). Genetic inactivation of DAGL-1α fully eliminates all form of endocannabinoid-mediated plasticity in the cortex, the hippocampus, the striatum and the cerebellum[57]. In contrast to the subtle effects of 2-AG, acute administration of exogenous cannabinoids markedly disrupts neuronal signalling and circuit dynamics (Box 1B). Consequently, THC and other exogenous CB1 receptor agonists decrease synchronised neuronal firing in the hippocampus and inhibit theta oscillations[58], LTP[59], and at the behavioural level, impair learning and memory.

Box 1 *The Molecularneuropharmacology of cannabinoids*

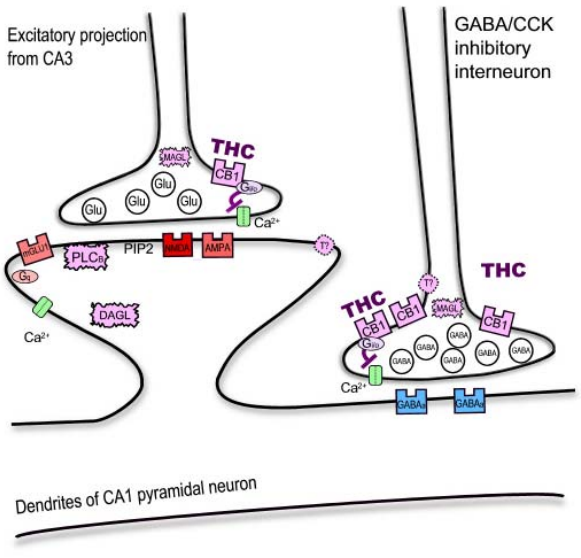
A) In area CA1 of the hippocampus, pyramidal neurons synthesize and release the endocannabinoid, 2-arachadonoylglycerol (2-AG); which acts at CB1 receptors on adjacent nerve terminals. Synthesis of 2-AG is driven by stimulation of metabotropic glutamate receptors (mGlu1) or by Ca^{2+}entry via voltage-operated channels. Compelling pharmacological evidence indicates the existence of an, as yet uncharacterised, eCB bi-directional transporter (T?). Consistent with a retrograde mode of action, the synthetic enzyme sn-1 diacylglycerol lipase (DAGL) is localised in dendritic spines, whilst 2-AG undergoes catabolism by monoacylglycerol lipase (MAGL) in pre-synaptic terminals.



Endocannabinoid dependent plasticity is expressed pre-synaptically as a, transient (5-30s) or prolonged (>1h), reduction in neurotransmitter release. Compared to excitatory terminals, GABA/CCK terminals in the hippocampus express more CB1 receptors and are more sensitive to cannabinoids. In CA1, it has been shown that locally released 2-AG depresses GABA-ergic inhibition, thereby facilitating long-term potentiation (LTP) at adjacent glutamatergic excitatory synapses. CB1 receptors on glutamatergic terminals might serve to limit the extent of 2-AG synthesis and arrest progression to seizures and excitotoxicity.

B) Exogenous cannabinoids such as Δ^9-tetrahydrocannibinol (THC) disrupt rather than mimic the subtleties of the eCB system in the hippocampus. THC inhibits long-term-potentiation of CA3-CA1 synapses and impairs learning and memory.

(*AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; DAG, diacylglycerol; Gi/o and Gq, G-proteins; IP3, inositol triphosphate; NMDA, N-methyl D-aspartate receptor; PIP2, phosphatidylinositol (4,5)-bisphosphate; PLC-β, phospholipase C-β.*) *Adapted from* [27]



Plant Derived Cannabinoids

Cannabis sativa contains over 60 different *phytocannabinoids*, of which only THC, cannabidiol (CBD) and Δ-9-tetrahydrocannabivarin (THCV) have been studied in any detail[60]. Unsurprisingly most research has focussed on THC, given it's dramatic effect on the human psyche.

Δ-9-Tetrahydrocannabinol

THC elicits a number of pharmacological effects, which are dependent upon dose. In some cases, responses can be biphasic. For instance in rodents and dogs relatively low doses of THC can stimulate motor activity, whereas high doses can induce catalepsy[61] Similarly, low doses of THC tend to be associated with an anxiolytic effect in animal models, whereas high doses can be anxiogenic[62]. Finally, in animal models, low doses of THC can elicit conditioned-place preference (CPP, a marker of 'addiction') whereas higher doses elicit aversion[63].

The pharmacology of THC covers a plethora of responses, some of which are therapeutically useful and others detrimental or unwanted. In the category of detrimental effects, it is well established that THC can impair short-term memory (the ability to register and recollect information)[61], and working memory/executive function (the ability to hold and manipulate information on-line)[64]. Acute psychosis, anxiety and panic can occur following THC[61].

Beneficial effects include analgesia, which probably stem from the expression of cannabinoid receptors in peripheral nerve fibres, dorsal roots, the spinal dorsal horn and the peri-aquaductal grey[65, 66]. Remarkably, existing analgesics such as acetominophen (paracetamol), appear to act via the endocannabinoid system[57]. For example, the CB_1 receptor antagonist SR141716A (Rimonabant), completely

abolishes the analgesic activity of paracetamol[67]. THC also has anti-inflammatory properties[68].

THC and other CB_1 agonists can stimulate appetite and food intake[69], probably via CB_1 receptors in the hypothalamus and the modulation of appetite mediators such as ghrelin, leptin and peptide YY (PYY)[70]. Sold as Marinol (Solvay Pharmaceuticals), THC has been approved by the U.S Food & Drug Administration (FDA) for the treatment of anorexia in AIDS patients. The license also covers the use of Marinol as an anti-emetic in patients undergoing chemotherapy.

Many patients suffering from multiple sclerosis (MS) patients report that their symptoms respond to cannabis. This prompted Baker and colleagues to investigate the efficacy of THC (and other CB_1 agonists) in an animal model of MS. They found that CB_1 agonists ameliorated the tremor and spasticity in diseased mice[71]. This line of reasoning was developed and a cannabis extract sold as Sativex (GW Pharmaceuticals) containing THC and CBD in a 1:1 ratio, delivered by oromucosal spray, has now been licensed in the UK for the treatment of severe spasticity in multiple sclerosis. Contraindications are a personal or family history of psychosis or other severe psychiatric disorder.

Cannabidiol

Cannabidiol lacks the dramatic effects on the psyche that are associated with THC[72]. In stark contrast there has been interest in CBD as a possible anti-psychotic and anxiolytic medication[73]. Animal work in the 1970's showed that CBD had anti-convulsive properties[72]. Safety and tolerability were established, permitting a trial in patients with refractory temporal lobe seizures, and positive outcomes were

reported[74]. Since the early 1980's there have been reports that CBD ameliorates anxiety in animal models[72] and recent human work suggests that CBD may be effective in social phobia[75].

CBD displays the signature of an anti-psychotic molecule in of animal models. To date it has been reported that CBD has efficacy across the standard models, involving the administration of apomorphine[76], amphetamine[77] or NMDA-channel-blockers[78]. Similar to clozapine, (but not haloperidol) CBD achieves this without eliciting motor side-effects (catalepsy)[72]. Experimental studies in man have also shown that CBD blocks the pro-psychotic effects of L-DOPA[79]. The first trial of CBD in schizophrenic patients reported equal efficacy with the established anti-psychotic amisulpride. Further work and confirmation is required however as numbers were small (i.e. the study was underpowered to detect a difference between CBD and the comparator) and the follow-up period, (4 weeks) was relatively short[80].

The receptor pharmacology of CBD remains enigmatic. Despite low affinity for the CB_1 and CB_2 receptors, low doses of CBD can antagonise cellular and tissue responses to CB_1/CB_2 agonists[81]. A plethora of receptor/enzymatic mechanisms have been reported for CBD including: antagonism of the orphan receptor GPR55, inhibition of adenosine re-uptake, inhibition of the cellular uptake and metabolism of anandamide, modulation of immune mediators and agonism at 5-HT_{1A} receptors[81].

THC & CBD in cannabis products

It is unique that a plant should harbour a component which appears to be pro-psychotic (THC) as well as a molecule with putative anti-psychotic properties (CBD). Most cannabis work in the context of psychosis/schizophrenia has focussed on THC, and the apparent rise in THC content found in sinsemilla [skunk], which is cultivated

indoors by high intensity methods. But some have suggested that CBD content may be an additional factor in determining the psychotogenicity of an individual cannabis strain[82]. Some evidence to support this came from South Africa where there was a cluster of acute psychotic presentations to emergency departments in people smoking a strain of cannabis which lacked CBD[83]. In broad agreement, experimental work from a Brazilian group in the early 1980's showed that CBD could antagonise the anxiogenic properties of THC.

It is well documented that the THC content in illicit cannabis products has increased. Work by Potter and by King in the UK indicates that modern products arising from indoor cultivation of selective strains contain approximately 3x as much THC by weight, compared to traditional products[84]. Whereas THC content has increased, CBD appears to have decreased markedly, and is often undetectable in illicit products. Sinsemilla or skunk (high THC content: low CBD content) is now believed to dominate the UK cannabis market, and Potter's work in particular provides strong evidence that this is the case[84]. If it is true that CBD antagonises the psychotomimetic properties of THC, then the lack of CBD in sinsemilla has implications for mental health in the community.

Cannabis and Schizophrenia.

The first link between cannabis and schizophrenia emerged in 1987 from a longitudinal study of over 45,000 Swedish conscripts. It was found that men who had smoked cannabis by the age of conscription had double the risk of schizophrenia in the ensuing 15 years[85]. (Individuals who had smoked cannabis on at least 50 occasions by recruitment were 6x more likely to develop schizophrenia within 15-

years). In 2002 a further analysis of the Swedish data (now involving over 50,000 subjects), ruled out the possibility that the association was due to other psychoactive drugs[86]. Another seven cohort or general-population studies have reported similar findings, and have been extensively reviewed[28, 87, 88].

The association between cannabis and psychosis is now generally accepted, given the consistency of findings from numerous epidemiological surveys. Overall, meta-analysis suggests that cannabis use increases the odds of developing a psychotic disorder by ~ x2[28]. Although the consistency between studies is in keeping with a causal effect of cannabis on psychotic disorder, other non-causal explanations are feasible – specifically; confounding and reverse causation ("*The self-medication" hypothesis*")[89]. The seven population studies outlined above made attempts to adjust for potential confounders such as stimulant use or childhood adversity, and found that associations between cannabis use and psychosis persisted, although the strength of the association typically decreased. However it is feasible that there are residual confounders, which were not accounted for. There is little evidence to support an explanation based on reverse causality, as most of the longitudinal studies excluded subjects with psychotic symptoms at baseline, or adjusted for symptoms at baseline[89].

Of course, the vast majority of people who use cannabis do not go on to develop a psychotic disorder, and there has been considerable interest in identifying which factors confer vulnerability. This effort has been underpinned by the conceptualisation of clinical psychosis/schizophrenia as a complex disorder, in which a constellation of risk-factors (environmental, genetic) interact to determine outcome, similar to the case with coronary heart disease for example. Amongst putative vulnerability factors, age of cannabis use onset (earlier = higher risk) has received empirical support[90]. There is also evidence suggesting that a history of trauma in childhood is a vulnerability

factor; Three studies found that the presence of childhood trauma and cannabis increased the risk over and above that posed by either factor in isolation[91-93]. Regarding vulnerability genes, an influential paper reported an interaction between a functional single nucleotide polymorphism in catechol-o-methyltransferase (COMT) and cannabis use, in conferring risk for psychosis[94]. An experimental study provided broad support[95] but a subsequent epidemiological study did not find an interaction. More consistent evidence has emerged to support an interaction between cannabis use and variation in the gene coding for Rac-alpha serine/threonine protein kinase, AKT1[96, 97].

THC & Acute Psychosis

In the last decade D'Souza and colleagues conducted a number of studies in which THC was administered to volunteers by the intravenous (IV) route. Using instruments designed for rating schizophrenic patients, it was found that THC (2.5mg or 5mg) could evoke time-limited positive psychotic symptoms and negative symptoms in otherwise healthy participants[98]. THC was also given to stable, medicated schizophrenic patients, which led to an acute exacerbation of psychotic symptoms in 75-80% of cases[99]. In healthy participants (and patients), cognitive performance was poorer under THC conditions, and impairments were said to be similar to the cognitive deficits in schizophrenia.

Psychoactive Drugs: Mechanism(s) of Action

The majority of psychoactive drugs recognise and bind to specific receptors on the surface of CNS neurons, and this is the case for THC. Drug binding stabilises the receptor protein in a particular conformation, which provokes a change in the neuron.

Typically, the binding process alters an internal signalling cascade or changes the electrical properties of the neuron[100].

Over the past 75 years, pharmacology has succeeded in characterising the components of such events at increasingly exquisite levels of detail: Receptors have been isolated, cloned, sequenced and re-engineered. Insight has accumulated about their 3-dimensional structure, their dynamics and how they are regulated within the cell. The same is also true of the components which make up the various downstream, intracellular signalling cascades, the ion-channels responsible for electrical signalling, and the uptake proteins which clear endogenous neurotransmitters from the synaptic cleft.

And yet our knowledge of how psychoactive drugs (of any class) elicit their effects on the mind and behaviour remains partial. Moving beyond the nanoscale - (the *small-world* of receptors and channels), and the micro-scale (the *world* of dendritic spines, synaptic boutons and individual neurons) - to the level of circuits, both local and long-range, the certainty of our knowledge diminishes rapidly. Only when we approach the level of the mind/behaviour does confidence in our statements return, and we talk about a drug being an anxiolytic or a sedative for example. The gulf is between events at the *small-world* scale and events at the level of the mind/behaviour, specifically in the area that describes the organisation and dynamics of neuronal ensembles/ circuits. It is self-evident that a complete account of the mechanism of action for *any* psychoactive drug necessitates detail at all levels.

To make this clearer, contrast this situation with an example from peripheral (non-CNS) pharmacology. Atropine elicits tachycardia. In this case, there is a consistent

and complete explanation, which covers all levels of organisation, proceeding from the nano-scale (*competitive antagonism of cholinergic M_2 receptors on the surface of atrial pacemaker cells, leading to the suppression of inhibitory trans-membrane potassium currents and swifter depolarisation*), to the micro-scale (*individual cells within the pacemaker reach their firing-threshold earlier and discharge action-potentials more frequently*) to the scale of local circuits (*cells making up the pacemaker population synchronise their action-potentials at a faster rate*) and finally to the global scale (*transfer of excitation through the conductive and contractile tissue of the heart at a higher frequency*)[101].

In contrast, in the CNS, it can be difficult to know where a drug acts to produce a specific psychological/behavioural effect. This is particularly true for drug-effects on ubiquitous, distributed systems, and the endocannabinoids fall into this category. Thankfully some psychological/behavioural effects are less challenging. For instance, if a drug inhibits fear conditioning, a wealth of evidence would direct investigations to synapses within the basolateral amygdaloid nuclei[102]. A drug effect on episodic memory would direct us to the hippocampal complex[58]. In the case of drug-elicited psychosis, the task is more difficult. Some of the reasons for this are outlined briefly, not to renounce the possibility of ever understanding the neurobiology of psychosis (drug-elicited or otherwise), but to bring the difficulties into the open. Before then however, it is important to clarify what is meant by the term "psychosis".

At the level of the mind, what is meant by "psychosis"?

Our modern understanding of psychosis was shaped by German academic psychiatry in the early 20th century. Both the DSM and ICD classification systems are structured according to the ideas of Karl Jaspers (1883-1969) and other leading figures from the

'Heidelberg school' of psychiatry. Influenced by continental philosophy (Husserl in particular), Jaspers adapted the phenomenological method for the psychiatric clinic. Phenomenology aims at clear, unambiguous descriptions of subjective experience, (devoid of theoretical 'baggage'). Jaspers' method involved entering the mindset of the patient, in as open, non-judgemental and impartial a manner as possible, whilst prioritising the *forms* through which consciousness manifests (e.g perception, ideas, will) over any over particular details about the c*ontent*[103].

Psychotic 'symptoms' include hallucinations (*the perception of objects which do not exist in reality*, *via any of the sensory channels*), and delusions (*demonstrably false ideas held with an unshakeable conviction, which fundamentally re-orientate one's way of being in the world*). A third form of psychotic symptom is less easily reduced to a disorder of perception or a disorder of ideation. These are the ipseity disturbances ("ipse"- self), in which the basic*, taken-for-granted* distinction between an internal self and the external world is blurred: Some patients have the experience that their own thoughts are no longer private, or perhaps that their thinking, will, movement or emotions are under the direct control of an external agency. The term "*breakdown of ego boundaries*" is sometimes used as a shorthand for this type of symptom[104] (2.1).

The organic basis of psychosis, (drug-elicited or otherwise)

A longstanding issue is whether the various psychotic experiences can be explained by neurological events at a *single anatomical locus*, given that psychosis can 'reveal itself' in an array of forms: via perception (*the input-channels*), thinking, the emotional mind, the sense of self (the ego), and the *output-channels* (movement and speech) [105]. In Husserlian terms 'psychosis' is not an '*essence*', '*beyond which one*

can make no further reductions', but [too] readily splits into 'purer' phenomena. Therefore, does it make sense to 'localise' psychosis [a mixture of forms] to a single anatomical locus? (A2.2).

'Special loci'

Nevertheless over time, various brain regions have been proposed to be *fundamental* in psychosis/(schizophrenia), including the hippocampus[106], the thalamus[107], the pre-frontal cortices[108], the higher auditory cortices[109], the ventral-striatum/*limbic*-striatum/*associational*-striatum[110], and the ventral-tegmental area/A10 dopamine cell group[111]. By necessity in any *single locus account,* the candidate *region/structure* would be required to be involved in the processing of diverse mental forms, ideas as well as perceptions for example. A *locus* might then be better described as a '*hub*'. Whatever the terminology, the *apparent problem* of accommodating diverse mental forms within a single region/structure appears to disappear.

Connectivity Hypotheses I

Other accounts have assumed the importance of particular regions/structures but have also stressed the importance of *connections* [between CNS regions]. Within this category of hypothesis, the following have been highly influential; frontal⇔temporal 'disconnection'[112]; over-activity in the A10 dopamine -> ventral-striatal 'reward/reinforcement/salience' pathways[113] (Box 2 *Classic studies*); and 'dysmetria' in a distributed network involving the frontal cortices, thalamus and cerebellum[114].

Connectivity Hypotheses II

A third family of hypothesis has become popular in the last few years. Here the spatial dimension, the issue of anatomical location, is less important. The critical factor is

time, specifically the organization of groups of neurons in time[115, 116]. To understand why they have become popular, it is necessary to take a [brief] detour into recent neurophysiology. We can set the stage by asserting that in health, the 'normal' operations of neuronal circuits/assemblies are the foundation of 'normal' conscious experience [perception, ideation, will and so forth][117-119] (A2.3). Similarly, 'abnormal' circuit/assembly operations are the foundation of 'abnormal conscious experience [hallucinations, delusions, ipseity disturbance etc.] [115, 116, 120]. It has been proposed that the content of conscious experience [regardless of form] arises from the *synchronous* firing of individual neurons, either locally [at the sub-millimeter level] or across regions [centimeter level][121-123]. *Synchronicity* 'requires' a metronome, (a tempo, a 'clock'), and it appears that the CNS utilizes neural oscillations (rhythms) for this purpose[124] (A2.4).

From about 1993 onwards, rhythms in the theta, gamma and other *frequency bands* became more than just 'markers' of particular stages of sleep -there was a paradigm shift in neurophysiology. Terms such as '*binding-by-synchrony'* and '*coherence-in-the gamma-band*' emerged as '*explanations*' for perception, thinking, attention [even 'consciousness' itself][118, 119] (A2.5). By the new millennium, it was realized that this list of higher faculties was *exactly* the same as those affected by major mental illness, and reports of '*abnormal*' coherence/synchronicity/oscillations/rhythms in schizophrenia began to appear[115, 116]. Some have suggested that the rhythm disturbances in schizophrenia arise because of histopathology in a specific type of GABA '*pacemaker*' neuron[125].

THC psychosis: Mechanism(s)

The small world

There is little doubt that THC psychosis begins with stimulation of central CB_1 receptors, since potent CB_1 antagonists inhibit the central effects of THC[126]. The CB_1 receptor is found at high density in the prefrontal and association cortices, the anterior, mediodorsal, and intralaminar thalamic nuclei, the hippocampal complex, amygdala, entorhinal cortex, basal ganglia, substantia-nigra pars-reticulata, and cerebellum[40, 127, 128].

Exogenous CB_1 agonists cannot be expected to mimic the subtleties of endogenous cannabinoid signalling such as spike-timing dependent LTD (A2.6). Endocannabinoid physiology is characterised by controlled local synthesis, release, uptake and swift metabolism[27]. So perhaps a safe assumption is that THC disrupts the normal functioning of the endocannabinoid system (in the same way that ketamine disrupts glutamate signalling or amphetamine alters monamines). For example, given that the endocannabinoid system is involved in setting the strength of synaptic connections (*hence*: learning and memory), it is intuitive that the amnestic properties of THC stem from disruption of normal endocannabinoid physiology.

The systems level

Two candidate mechanisms have been proposed to account for the pro-psychotic effects of THC, excess striatal dopamine release[27] and abnormal neural oscillations[129, 130].

Dopamine has been at the heart of psychosis/schizophrenia for over forty years[12], although interest waned (temporarily) in the early to mid 1990's, with the rise of serotonin and glutamate-based accounts. But a study by Laruelle and colleagues in

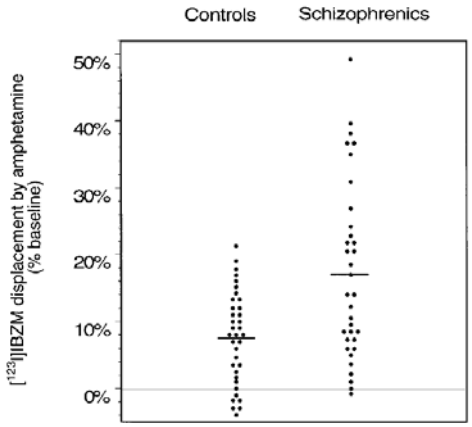
1996 rekindled the dopamine hypothesis[131]. Using single-photon-emission-tomography (SPET) it was shown that, compared to healthy controls, schizophrenic patients release greater amounts of dopamine into the striatum following the administration of IV amphetamine, and those patients showing the highest release experienced a transient relapse of psychotic symptoms (Box 2: Classic Studies).

Here a similar methodology was applied for THC (study 2). It was hypothesised that THC would *also* induce excess striatal dopamine release and, in doing so, give rise to psychotic symptoms. The rationale was based on animal work. Cannabinoid CB_1 agonists increase dopamine cell firing, elicit burst-firing, and increase the release of dopamine at terminal fields in the striatum[132-136].

Animal work provided the rationale for study 3, the effect of THC on neural oscillations measured by electroencephalogram (EEG). Cannabinoid CB_1 agonists have been shown to disrupt neural oscillations[58, 129]. Indeed CB_1 agonists have become a useful tool, because they disrupt network synchrony without impinging on the firing rates of individual cells[137]. The assumption of course is that neural oscillations are important for 'normal' mental operations, but as outlined above (and in appendix 2.4) this assumption is not wholly devoid of experimental support. What is without any doubt however is that neural oscillations have an intimate relationship with the spiking behaviour of individual neurons within the tissue (Box 2: Classic Studies).

BOX 2: *Classic Studies*

A) Dopamine had long been associated with schizophrenia. In the 1990's a series of intravenous amphetamine-challenge studies measured dopamine release, as indexed by the displacement of the D2-radiotracer [^{123}I)IBZM. The major finding was that, *post-amphetamine*, schizophrenic patients showed greater striatal dopamine release than healthy controls.

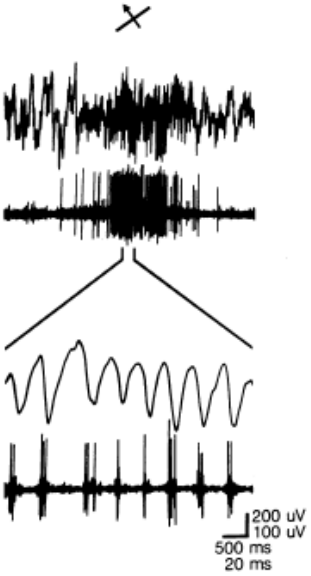


In the patient group, positive psychotic symptoms as rated by the PANSS increased following amphetamine (mean increase 3.0 points). The magnitude of positive symptoms was related to the extent of dopamine release (r^2=0.3, p<0.001). Laruelle & Abi-Dargham (1999) *J Psychopharmacology* **13**:358.

B) Until recently EEG research meant event-related-potentials (ERP's), in which the '*real*' signal (the ERP) was extracted from the '*noise*' by averaging many trials. But in 1989 there were the beginnings of a paradigm shift. Gray & Singer were studying electrical responses in the primary visual cortex of anaesthetised cats: but somewhat unusually they didn't use patch or intracellular electrodes, the most '*refined*' methods of the day. Instead they measured the local field potential (equivalent to the EEG signal) *as well as* spiking behaviour (action-potentials) from numerous individual neurons.

They found that visual stimuli (moving bars) evoked a *burst* of oscillatory activity and a burst of spikes. The 'game-changing' observation however, was that spikes (action-potentials) were phase locked to the trough of the oscillating (*40Hz, gamma*) rhythm (but not to the stimulus).

In the '*flood*' of papers that followed, a fundamental insight was that neurons reacting to distinct features of the same physical object *synchronised* their spikes with high precision.



1. Field & Spikes respectively, at low temporal resolution.
2. Field & Spikes respectively, at high temporal resolution. Gray & Singer (1989) PNAS ***86****:1698).*

The effect of Cannabidiol on THC-elicited psychosis

In stark contrast to THC, cannabidiol (CBD) is reported to have anti-psychotic properties in animal models and in schizophrenia[73, 80]. The relative concentration of CBD in modern 'high-potency' products (sinsemilla) is low[84]. Some have speculated that the absence of CBD in sinsmilla endows this product with increased risks for mental health[82]. Laboratory studies are ideal for testing this idea, because pure cannabinoid preparations can be administered under controlled conditions. Hence, in study 4 (a pilot) and study 5, it was hypothesised that pre-treatment with CBD would inhibit THC-elicited psychosis.

CHAPTER 1

STUDY 1: The effects of intravenous THC in healthy Volunteers

BACKGROUND

Since the 19th century there have been descriptions of acute schizophrenia-like *positive* psychotic symptoms in healthy subjects who had taken cannabis or been given THC[16-19]. The adverse effects of THC on memory are also well established. There remains controversy on whether cannabis mimics the *negative* symptoms of schizophrenia (e.g. loss of drive and motivation, reduced emotional expression, poverty of thought).

Several studies have found that, amongst schizophrenic patients, the use of cannabis is associated with *less* negative symptomatology[138-140] and one proposal is that patients self-medicate to alleviate negative symptoms[138, 140]. It has also been reported that cannabis-using healthy subjects exhibit less negative syndrome schizotypy compared to drug-free controls[141, 142]. Few laboratory studies have investigated the acute effects of cannabis/THC on the negative dimension. In contrast to the above, D'Souza and colleagues reported that IV THC *increased* negative symptoms in stable schizophrenic patients and in healthy controls[98, 99]. The authors did, however, acknowledge that the rating scale used may have been unable to distinguish true negative symptoms from sedation[98].

AIM

To confirm the pro-psychotic effects of THC, quantify the impact of THC on cognition and mood; and to explore if there are relationships between these primary outcome measures.

METHODS

Design

The psychological properties of IV THC (2.5mg) were investigated in an experimental study utilising a within-subject, double-blind, placebo-controlled design. Participants attended 2 experimental sessions, at least 2 weeks apart, in which either THC or placebo was given in a random, counterbalanced order.

Participants

Healthy male participants between 21 and 50 years who fulfilled entry criteria. Participants were asked to avoid alcohol and drugs for 24 hours before, and to abstain from driving for 24 hours after, experimental sessions. Participants were followed up by telephone the following day and a small monetary re-imbursement was made at the end of their involvement in the study. Participants (21-50) were required to have previously taken cannabis on at least one occasion; to score <15 on The General Health Questionnaire (GHQ-12)[143]; and be willing to provide written informed consent. Exclusion criteria were a history of mental illness, substance dependence (excluding nicotine), current or past severe medical disorders or a history of major mental illness in a first degree family member. Alcohol and drug dependence were excluded using the Michigan Alcohol Screening Test (MAST)[144] and the Drug abuse Screening Test (DAST)[145].

Pharmaceuticals

Dronabinol (THC) was supplied by THC Pharm GmbH (Frankfurt am Main, Germany) and prepared as (1mg/ml) vials for intravenous injection by Bichsel

Laboratories, (Interlaken, Switzerland) according to the method of Naef and colleagues[146]. Placebo and active vials were identical in composition (except for THC) and identical in appearance. After dilution in normal saline, preparations for injection contained 2.5% (v/v) ethanol absolute. Previous studies of the effects of IV THC have utilized doses ranging from 2 to 5mg which approximate the levels of THC from smoking a standard cannabis 'joint' containing 1-3.5% THC (16-34mg)[98].

Outcome Measures

Psychological assessments and self-rated scales were administered at baseline (30 minutes prior to injection) and at 30, 80 and 120 minutes following the final injected pulse. Participants were instructed to complete rating scales as applied to the present moment.

1.Psychotic Symptoms

Instruments: The PANSS and the CAPE-state.

THE PANSS (The positive & Negative Syndrome Scale)[147]

The PANSS is a 42-item scale designed to measure the extent of psychotic symptoms in patients with schizophrenia. Items are rated (1-7). The 3-factor version includes a positive, negative and general dimension.

The CAPE-state (Community Assessment of Psychic Experiences-state version)

The CAPE-state is a validated 42-item self-reported questionnaire, derived from The Peters Delusions Inventory, which generates a positive, a negative and a depressive dimension score[95]. In the studies here the CAPE-state frequency score (0-never, 1-sometimes, 2-often, 3-nearly always) was collapsed to a yes/no response option

2.Affect

Instrument: The UMACL

The UMACL (University of Wales Mood Adjective Checklist)[148]

Three dimensions of affect are measured - hedonic tone, energetic arousal and tense arousal (Matthews et al. 1990). It has been suggested that a 3-dimensional model of affect with separate *pleasure-displeasure*, *awake-tiredness*, and *tension-relaxation* dimensions provides a more informative description of core affect (compared to 1-dimensional and 2-dimensional models) and fits better with experimental data[148]. For each dimension, subjects rated their level of agreement with four emotionally positive and four emotionally negative adjectives. The total score in each dimension was calculated by subtracting negatively valenced from positively valenced items. For each subject the total score in each dimension was a value between –12 and +12.

3.Cognitive Testing

Cognitive assessments began 10 minutes post-injection. The order of tests was consistent across both sessions (1. RAVLT-immediate recall, 2. Digit-span, 3. Verbal fluency, 4. RAVLT-20 min recall, 5 The N-back task, 6. The Baddeley reasoning task; Appendix 1). Cognitive testing was complete within 45 minutes post-injection.

The Rey Auditory Verbal Learning Task (RAVLT)

The standard administration format of the RAVLT was utilised. Immediate recall of a 15-word list was assessed over 5 trials. Two different, but equivalent word lists were utilised. Free-recall was assessed following a 20-minute delay.

Digit Span

The Digit Span task evaluates the capacity of attention and working memory. Participants were tested in their immediate recall of a sequence of digits; and given 2 attempts at each level of difficulty. In the reverse digit span condition, participants were required to recall the sequence in the reverse order.

The N-Back task

The n-back procedure has been used extensively to measure human working memory performance[149]. Participants were required to monitor a series of 20 standard playing cards for 2 seconds per card. They were required to recall both the suit and number of the card n-integers back, where n=0-2 ("0-2 back"), in three consecutive sessions with increasing level of difficulty. Responses were scored correct/incorrect, giving a maximum score of 20 for each level of difficulty. The task requires continuous updating of information stores in the 1-back and 2-back conditions. In contrast the 0-back condition does not require *manipulation* of material in WM.

The Baddeley Reasoning Task

Participants were given 3 minutes in which to verify the truth of 32 logical statements containing 1 of 4 grammatical constructs such as; "B does not precede A….AB". The highest obtainable score was 32. The Baddeley Reasoning Task evaluates the performance of the central executive.

4.Pharmocokinetics

Blood (5mL) was collected at baseline and at 1, 5, 15, 30, 60 and 120 minutes post-injection, and stored at -70ºC until analysis for [THC] and its major metabolites. The samples were extracted using solid phase extraction (SPE). Cannabinoids were derivatized and measured using gas chromatography with mass-spectrophotometric detection (GCMS) as described previously[150].

Statistical analyses

Data was assessed for normality using Kolmogorov-Smirnov test statistics. Because of absence of variance in positive psychotic scores under placebo conditions, Friedman's Test, a non-parametric, repeated measures test was used to compare positive psychotic scores under THC and placebo conditions. Two-way repeated measures ANOVA was used to compare immediate recall in the RAVLT, with Trial (1-5) and Treatment (Placebo v THC) as within-subject factors. The Greenhouse-Geisser corrected *F*-ratio was used because there was a violation of sphericity. For the remaining cognitive tasks and the UMACL, differences between placebo and THC were compared using paired *t*-tests. Relationships between normally-distributed variables were analysed using Pearson's product moment correlation. Relationships between non-parametric variables were tested using Spearman's rank correlation coefficient. Significance was accepted at p-values<0.05

RESULTS

Of 24 volunteers, 22 attended both sessions. Participants were aged 28±6 years (mean±SD). Estimated lifetime exposure to cannabis ranged from 2 to ~1000 episodes. Self-reported last previous use of cannabis ranged from 12 hours to 10 years (mean±SD: 2 years, 3.3 years). In urine drug screens, one participant tested positive for THC, but this was the case prior to both experimental sessions, thus data was included in the main analyses. No participants tested positive for other common drugs of abuse (opiates, cocaine, amphetamines, methamphetamine, methadone, benzodiazepenes) at either session. Of 22 participants, 3 subjects were unable to complete all parts of the protocol. One participant experienced nausea during the THC arm. Another participant refused to participate with cognitive testing or complete self-rated scales under THC. Finally one participant experienced profound anxiety during the THC arm and requested 'rescue medication' (lorazepam 3mg) – and symptoms resolved completely within 30 minutes. Verbal reports of the IV THC experience are shown in Box 3.

THC induced positive psychotic symptoms

Scores on the PANSS positive subscale were increased from baseline following THC but not placebo administration (Friedman's χ^2=62, p<0.001). At 30 minutes post THC, PANSS positive scores had increased by a mean of 3.7 points (range 0-17), returning to baseline levels by 120 minutes (Figure 1.1). Similarly, subject-rated positive psychotic symptoms as measured by the CAPE-state increased from baseline following THC but not placebo (Friedman's χ^2=20, p=0.005). By 80 minutes post-injection, CAPE-state scores had returned to baseline (Figure 1.1). Investigator-rated (PANSS) and subject rated (CAPE-state) positive psychotic scores at 30 and 80

minutes post-THC administration were correlated (Spearman's rho=0.62, $p<0.001$). There was no relationship between positive psychotic symptoms, as measured by the PANSS and plasma concentrations of THC at 5 minutes, or 11-OH-THC concentrations at 5 minutes. Similarly, PANSS-positive scores following THC administration and $AUC_{0-\infty}$ were unrelated.

Participants who had taken cannabis more often in the past [estimated number of episodes] were less likely to exhibit positive psychotic symptoms as rated by the PANSS under THC conditions at 30 minutes post injection (Spearman's rho= -0.45, $p<0.05$).

THC induced negative symptoms

Scores on the PANSS negative subscale were increased from baseline following THC but not placebo administration (Friedman's $\chi^2=30.1$, $p<0.001$).). At 30 minutes post THC, PANSS positive scores had increased from 7.0±0.0 (mean±SD) at baseline to a peak of 7.7±1.3. Scores on the CAPE negative dimension were increased from baseline following THC but not placebo administration (Friedman's $\chi^2=25.3$, $p=0.001$). At 30 minutes post THC, CAPE negative scores had increased by a mean of 4 points, returning to baseline levels by 120 minutes post-injection (Figure 1.2). There was no relationship between peak negative symptoms as rated by the CAPE and the PANSS. Peak changes in CAPE-negative scores were not related to plasma concentrations of THC (AUC0-∞).

Box 3: Verbal Reports following IV THC 2.5mg

Participants' verbal reports were recorded. Selected responses are included here.

"*A feeling of great insight…I must write down this fantastic theory… of course it's all nothing*".

"*Every occurrence, cough, object, test, has deeper and connected meaning… All deliberate, planned…some sort of prank, to make a fool of people*".

"*It was like you guys were having a conversation outside, saying… 'the dude in there is off his rocker'* ".

"*It was threatening and sinister. I'm still a bit unsure of you. Your re-assurances sound insincere".*

"You know you were…[reading my mind]".

"I could hear your voice booming, outside the window".

"…there was a dissociation between movement and the will to move".

"There seemed to be a disconnect between the original idea of moving and the actual instruction to move."

"It feels like my legs are being controlled by strings from the ceiling. Yes… actual strings."

"I felt I was so mad I would not have been surprised to find out that I had been posturing."

" There's an incredible disconnection between thinking and saying something…"

" I wasn't sure, after saying something if I had even been talking… I thought I had just thought the words but not said them".

"…The key theme in hallucinations…you do not realise if you are saying these words out loud or just thinking it".

"It felt like my parietal lobe was talking. The thoughts would echo around the room".

(Responses were used to construct a questionnaire, See Appendix 1.)

Intravenous Δ^9-Tetrahydrocannabinol elicits positive psychotic symptoms

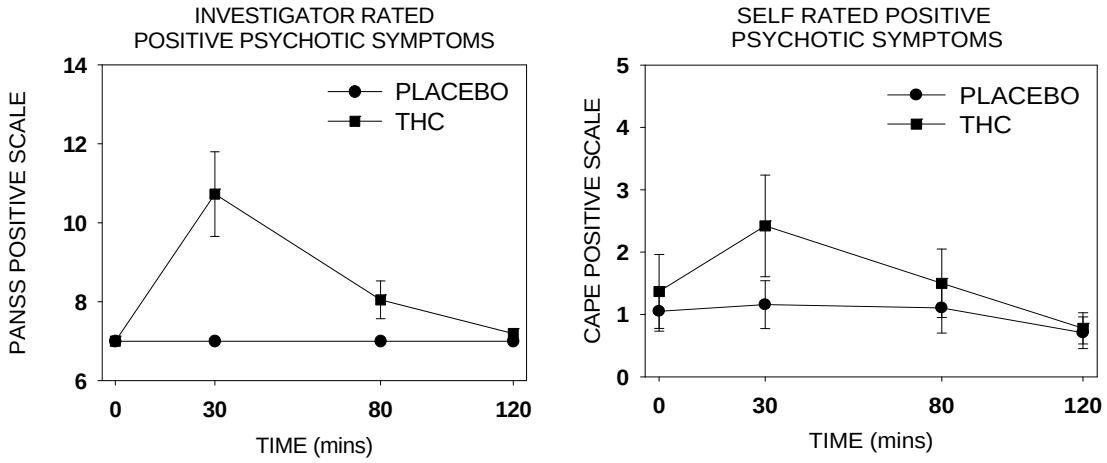


Figure 1.1. Following the administration of IV THC 2.5mg, healthy participants experienced positive psychotic symptoms (mean±s.e), whether rated by an external observer (The positive & negative syndrome scale [PANSS]; left panel) or according to scores on a self-rated scale (The community assessment of psychic experiences – state version [CAPE-state]; right-panel).

Intravenous Δ^9-Tetrahydrocannabinol elicits negative symptoms

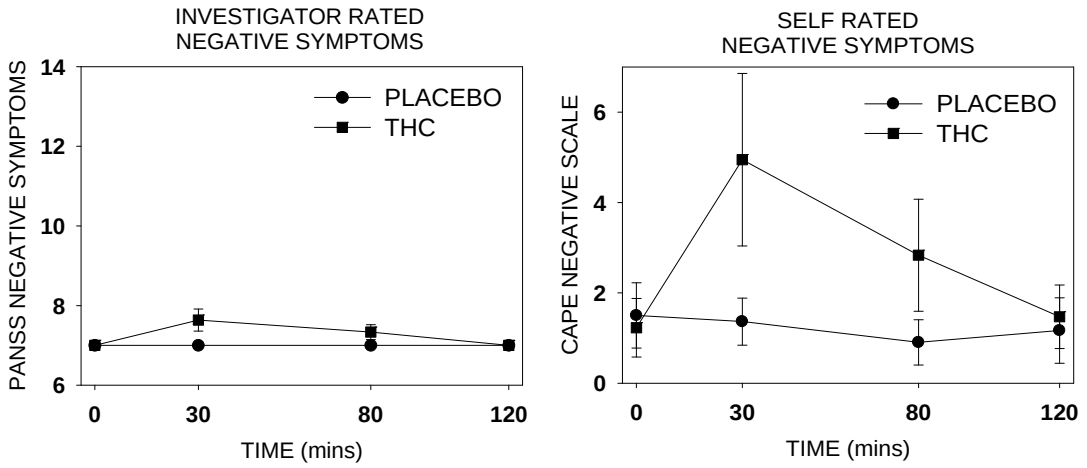


Figure 1.2. Following the administration of IV THC 2.5mg, healthy participants experienced negative symptoms (mean±s.e), whether rated by an external observer (The positive & negative syndrome scale [PANSS]; left panel) or according to scores on a self-rated scale (The community assessment of psychic experiences – state version [CAPE-state]; right-panel).

The Effects of IV THC on Core Affect

Hedonic Tone

IV THC elicited transient feelings of dysphoria. Under THC conditions, self-rated hedonic tone as measured by The UMACL, was decreased compared to placebo at 30-minutes post injection (THC, mean=4.3, *95% CI*= 2.9-5.8; placebo, mean=8.7, *95% CI*= 7.3-10.1, $t(17)=3.24$, $p=0.005$) and at 80 minutes post-injection (THC, mean=7.2, *95% CI*= 6.3-8.1; placebo, mean=9.3, *95% CI*=8.5-10.2, $t(15)=2.62$, $p<0.05$). By 120 minutes there was no significant difference in hedonic tone between THC and placebo conditions (Figure 1.3a).

Energetic Arousal

On the UMACL awake-tiredness dimension, THC induced feelings of tiredness at 30 minutes (THC, mean=0.3, *95% CI*= -1.8-2.4*;* placebo, mean=4.6, *95% CI*= 3.3-5.8, $t(17)=4.09$, $p=0.001$), at 80 minutes (THC, mean= -1.0, *95% CI*= -3.0-1.1*;* placebo, mean=5.5, *95% CI*= 4.3-6.7, $t(15)=4.74$, $p<0.000$) and at 120 minutes post-injection (THC, mean= -0.2, *95% CI*= -1.6-1.3 *;* placebo, mean=5.1, *95% CI*= 3.7-6.6, $t(9)=$, $p<0.01$) (Figure 1.3b).

Notably, there was no relationship between changes in self-rated sedation and the CAPE-negative score under THC conditions (Spearman's rho=-0.26, p=0.3).

Tense Arousal

On the UMACL tension-relaxation dimension, THC induced feelings of tense arousal at 30 minutes (THC, mean= -0.7, *95% CI*= -3.2-1.9*;* placebo, mean=

-7.3, *95% CI*= -8.5-(-6.1), *t*(17)= -3.93, *p*=0.001) and at 80 minutes post-injection (THC, mean= -3.3, *95% CI*= -5.7-(-1.0)*;* placebo, mean= -8.3, *95% CI*= -9.4-(-7.2), *t*(15)= -3.2, *p*<0.01).). By 120 minutes there was no significant difference in tense arousal between THC and placebo conditions (Figure 1.3c).

Notably, there was no relationship at 30 and 80 minutes post-injection between the degree of THC-elicited tense arousal and positive psychotic symptoms whether measured using the PANSS (Spearman's rho=0.19, p=0.30) or the CAPE-state (Spearman's rho=0.28, p=0.12).

The Effects of THC on cognitive performance

Working memory

Immediate recall under THC or placebo conditions (Treatment) was compared in successive trials A1-A5 (Trial) of the RAVLT (Fig 1.4). There was an effect of Treatment, *F*(1, 19)=16.6, *p*<0.005, and an effect of Trial *F*(2.71, 51.49)=85.3, p<0.000, but no Treatment*Trial interaction *F*(3.04, 57.82)=2.24, *p*=0.09).

Similarly, THC decreased performance in the forward (mean±SD: THC=7.0±1.2, placebo=7.8±0.9, *t*(18)=2.62, *p*<0.05) and reverse digit-span task (THC = 5.1±1.4, placebo= 6.2±1.1, *t*(18)=3.3, *p*<0.005) (Fig 1.5). Working memory deficits were unrelated to positive psychotic symptoms.

Episodic memory

There was a trend towards a difference in free recall (*at 20 minutes post-encoding*) between placebo (mean±SD: 11.1±3.3) and THC (9.7±3.5) conditions, *t*(17)=1.75, *p*=0.10 (Figure 1.4). Poorer episodic memory performance was related to higher PANSS positive scores (Spearman's rho= -0.4, p<0.05).

he Effects of IV THC (2.5mg) on Core Affect

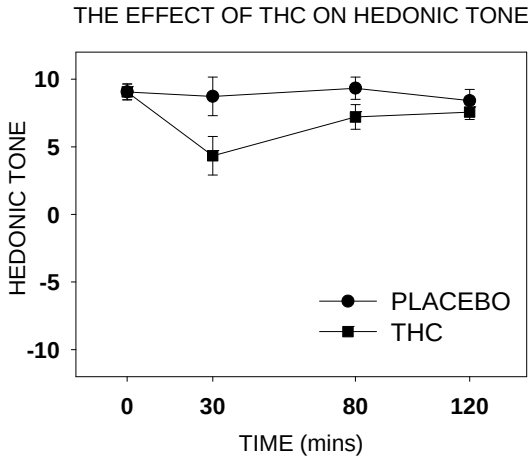


Figure 1.3a Mean scores of hedonic tone as measured by the University of Wales Institute of Science & Technology mood adjective checklist (UMACL) following intravenous THC (2.5mg). Error bars show ± 95% CI.

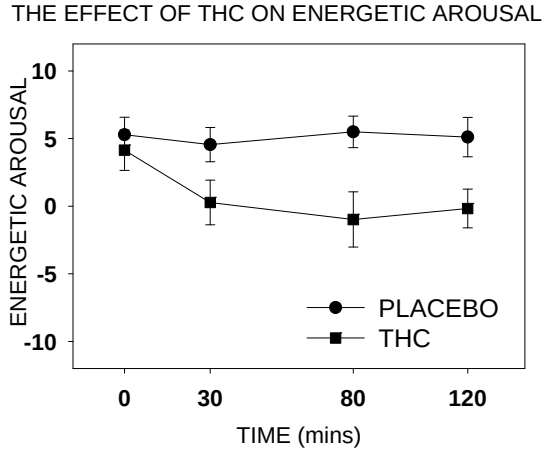


Figure 1.3b Mean scores of energetic arousal as measured by the University of Wales Institute of Science & Technology mood adjective checklist (UMACL) following intravenous THC (2.5mg). Error bars show ± 95% CI.

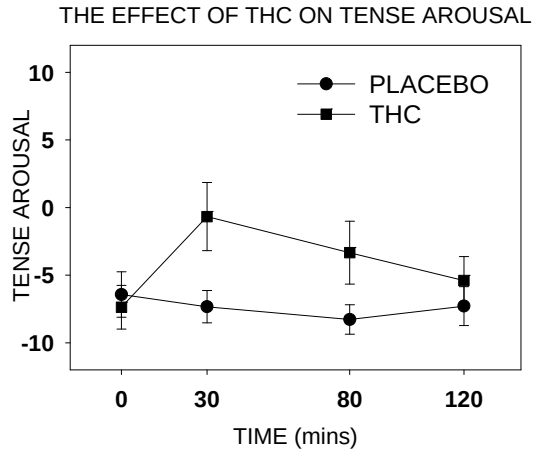


Figure 1.3c Mean scores of tense arousal as measured by the University of Wales Institute of Science & Technology mood adjective checklist (UMACL) following intravenous THC (2.5mg). Error bars show ± 95% CI.

Verbal fluency

Participants showed no difference in performance between THC (mean±SD: 19.3±3.2) and placebo (18.9±4.2) conditions, $t(15)=-0.3$, $p=0.7$ (Figure 1.6).

Executive function

Performance under THC or placebo conditions (Treatment) was compared in successive levels of difficulty (Level) in the N-Back task (Fig 1.7a). There was an effect of Treatment $F(1, 17)=7.1$, $p<0.05$ and an effect of Level $F(1.33, 22.5)=130.1$, $p<0.001$) but no Treatment*Level interaction. Performance in the Baddeley reasoning task under THC (mean±SD: 22.3±7.6) was significantly poorer than under placebo conditions (26.9±5.9), $t(15)=3.6$, $p=0.003$ (Figure 1.7b). Performance in the n-Back and Baddeley reasoning task were unrelated to positive psychotic symptoms.

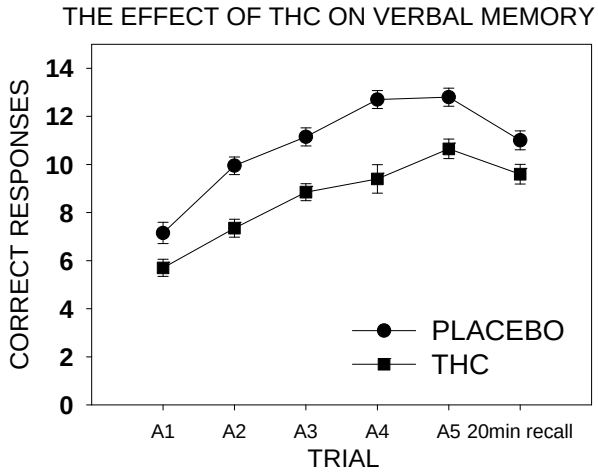


Figure 1.4 Performance in the The Rey Auditory Verbal Learning Task (RAVLT) following intravenous THC (2.5mg) or matched placebo. Mean±s.e scores are shown for consecutive trials of working memory (A1-A5) and for free recall at 20-minutes post-encoding.

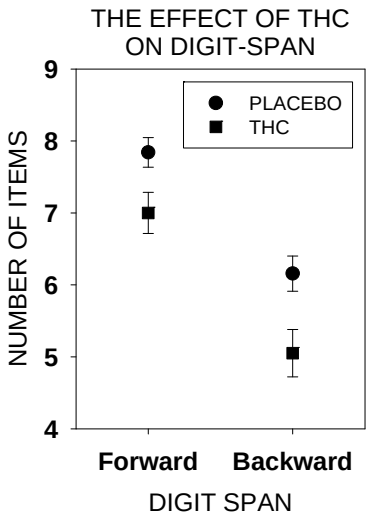


Figure 1.5 Performance on the digit-span task following intravenous THC (2.5mg) or placebo. Mean±s.e scores are shown for digit-span forward and backward.

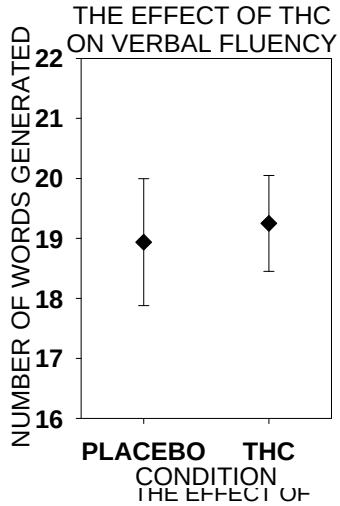


Figure 1.6 Performance on the verbal fluency task following intravenous THC (2.5mg) or placebo. Mean±s.e

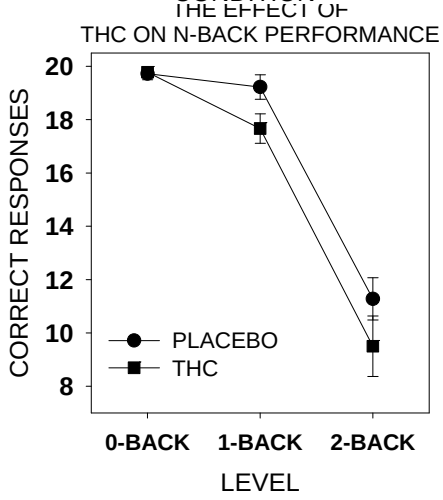


Figure 1.7a Performance on the N-back task following intravenous THC (2.5mg) or placebo. Mean±s.e are shown at increasing levels of difficulty (0-BACK → 2-BACK).

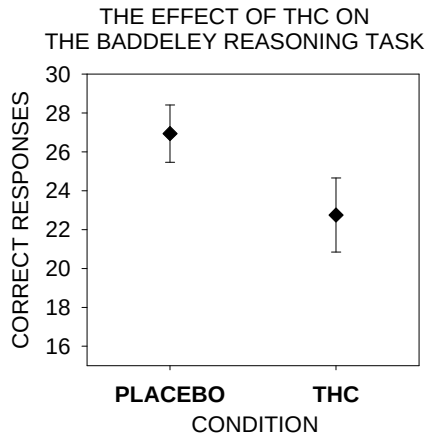


Figure 1.7b Performance on the Baddeley Reasoning task following intravenous THC (2.5mg) or placebo. Mean±s.e are shown. (Note; *Maximum score=32*).

Pharmacokinetics

Following IV THC (2.5mg over 5 minutes), plasma concentrations reached a maximum and then decreased rapidly over 15 minutes. Thereafter, concentrations decreased at a slower rate (Figure 1.8). At 1 minute post-injection the plasma concentration of THC ranged between 96.1 and 206.6 ng/mL (mean±SD: 176.7±33.7). Peak plasma concentrations of the main psychoactive metabolite of THC - 11-OH-THC - occurred at 5-minutes post-injection and ranged from 1.5 to 7.8 ng/mL (mean±SD: 4.4±2.1). THC area under the curve from time 0 to infinity ($AUC_{0-\infty}$) ranged from 1110.1 to 2995.4ng·min·mL^{-1} (mean±SD: 2047.3±423.7).

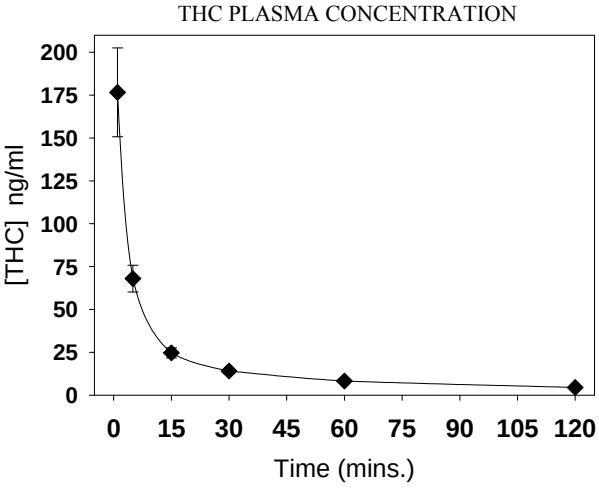


Figure 1.8 Mean plasma concentrations following the intravenous administration of THC (2.5mg). Error bars show ± 95% CI.

DISCUSSION

The observed plasma concentrations and time-course of THC in the present study are in a similar range to those from previous studies in which individuals smoked cannabis cigarettes. In a study of healthy males, mean plasma levels of THC immediately after 10 puffs from cannabis cigarettes containing 1.75% or 3.55% THC, were respectively 56.2 and 146.6 ng/mL[151]. Ramaekers and colleagues investigated the properties of high-potency marijuana cigarettes containing 13% THC in a sample of recreational cannabis users. The mean (SD) serum THC concentration at 5 minutes post-smoking was 93.6 (63.9) ng/mL[152]. In the present study, the mean (SD) plasma THC concentration at 5 minutes post injection was 68.0 (14.1) ng/mL.

Psychological responses to THC began during the redistribution phase (~5-10 minutes post injection) when the plasma concentration of THC had already fallen sharply (Figure 1.8). The majority of the psychological effects tapered off between 60 and 120 minutes post THC, despite the relatively small change in the absolute plasma concentration during that period (Figure 1.8). Thus in agreement with previous reports the onset, peak and termination of psychological responses to THC were not related to concurrent plasma concentrations[153].

Positive Psychotic Symptoms

There was wide inter-individual variation in psychosis scores. At 30 minutes post-injection, 50% of subjects had increases in the PANSS positive subscale score ≥ 4, and similarly, 47% of subjects endorsed positive subscale items from the CAPE-state. Under THC conditions there was a significant correlation between increases in PANSS-positive and CAPE-state positive scores at 30 and 80 minutes. This suggests that phenomena which were categorised as psychotic were likely to be 'true'

psychotic experiences. THC-psychosis as measured by the PANSS or the CAPE-state did not correlate with THC elicited anxiety suggesting that THC-elicited acute anxiety and THC-elicited acute psychosis are separable psychological effects. The most commonly endorsed items on the CAPE-state under THC conditions at 30 minutes post injection were Q.2 *Do you feel as if people seem to be dropping hints about you or saying things with a double meaning?*, and Q.30 *Do you hear your own thoughts being echoed back to you?*

Negative symptoms

IV THC elicited self-reports of schizophrenia-like negative symptoms as rated by the CAPE scale. The most commonly endorsed items at 30-minutes post-injection were, 1) *Do you feel that you are not much of a talker at the moment?* 2) *Do you feel that you are not very animated?* 3) *Do you feel that you are lacking in energy / motivation / spontaneity?* 4) *Do you feel that you experience few or no emotions at this time?* Self-reported negative symptoms were unrelated to sedation. Scores on the investigator-rated PANSS negative subscale were also increased under THC-conditions. However the effect size was numerically small and showed no correlation with CAPE-rated self-reports of negative symptoms. Whereas the presence of positive symptoms appears to be 'obvious' to participant and investigator alike, instruments such as the PANSS may be unsuited for quantifying experiences of negative symptoms in acute pharmacological studies.

Affect

As hypothesized, THC elicited pronounced (largely unpleasant) changes in core affect. Overall, participants were more likely to rate themselves on the UMACL as

dysphoric and anxious in the first hour following THC. (Anecdotally, many subjects gave verbal reports of feeling more relaxed following THC but in the majority of cases their verbal reports were in contradiction to their self-rated scores on the tense-arousal dimension of the UMACL and the overall clinical impression. Perhaps this reflects an underlying, widely-held assumption that cannabis has tranquilising properties.) Dysphoria and anxiety appeared to peak in the early stages following IV-THC and subsided quicker than subjective feelings of tiredness, which peaked later and were more persistent.

Animal and human work suggests that the effects of THC on anxiety is biphasic, with lower doses being anxiolytic and higher doses being anxiogenic[61]. The anxiety response observed here and in the study by D'Souza et al 2004 may be attributable to high plasma levels of THC. Clearly, the setting and drug-delivery method are far removed from the naturalistic way of taking cannabis and may have facilitated an anxiety response.

Cognitive Function: In the 30 minutes following THC administration, there were marked deficits in working memory and executive functioning and a trend towards impaired episodic memory, all of which are largely consistent with previous studies[64, 154]. In the sample here we found no suggestion of any relationship between the extent of positive psychotic symptoms and working memory/executive function. The extent of positive psychotic symptoms was related to impairments in episodic [hippocampal-dependent] memory however.

CONCLUSION

In a sample of healthy male subjects, a pure, synthetic IV preparation of THC elicited acute positive psychotic symptoms, negative symptoms, anxiety, dysphoria, working memory/executive deficits and subsequently, feelings of tiredness. Positive psychotic symptoms were neither secondary to high levels of anxiety nor a cause of anxiety.

CHAPTER 2

STUDY 2: The effects of intravenous THC on dopamine release within the striatum

BACKGROUND

Animal work has demonstrated that THC stimulates burst-firing of midbrain dopamine (DA) neurons and increases DA release at terminal fields in the striatum[132, 134, 155]. Given that excessive striatal dopamine has frequently been implicated in psychosis[156], it is feasible that THC-psychosis is mediated via excess striatal DA release.

HYPOTHESES

1. Compared with placebo, THC would elicit a decrease in specific [123I]-IBZM binding in the striatum (an index of DA release).

2. The magnitude of [123I]-IBZM displacement would show a relationship with positive psychotic symptoms.

METHODS

The study was carried out in the Institute of Nuclear Medicine, University College Hospital (UCLH), University College London, following approval from the South London and Maudsley Regional Ethics Committee and from the United Kingdom Administration of Radioactive Substances Advisory Committee (ARSAC).

Design

The psychological properties of IV THC (2.5mg) were investigated in an experimental study utilising a within-subject, double-blind, placebo-controlled design. Participants attended 2 experimental sessions, at least 2 weeks apart, in which either THC or placebo was given in a random, counterbalanced order.

Participants

Healthy male participants between 21 and 50 years who fulfilled
entry criteria as in study 1.

Pharmaceuticals

Dronabinol (THC) 2.5mg IV over 5-minutes, as in study 1.

[123I]-IBZM at 185MBq was obtained from commercial sources (GE Healthcare, Eindhoven). Participants were given potassium iodate tablets for thyroid protection as per UCLH protocols (170mg/d for 5 days, starting 2 days prior to the scan).

SPET Procedure

Previous work has shown that following a bolus injection, specific [123I]-IBZM binding reaches a plateau by 45-60 minutes[157]. Verhoeff et al. (1991) advised that optimal scan time was between 60 and 150 minutes[158]. Here, participants received a bolus dose of [123I]-IBZM (185MBq) two hours prior to the onset of a 120 minute duration SPET scan. Forty-five minutes into the scan, participants were administered either IV THC (2.5mg) or placebo in a randomised, counterbalanced order. In both sessions, the first 0-45 minutes was used as a baseline. This permitted 30 minutes for the establishment of a new [123I]-IBZM *pseudo*-eqilibrium. Scanning data collected between 75-120 minutes were the defined drug/placebo blocks.

SPET Data Acquisition

SPET data was acquired using a triple detector Prism 3000XP (Philips Medical Systems, Cleveland, Ohio) camera with an ultra-high resolution low-energy fan-beam collimators. The images were acquired in a 128x128 pixel matrix. The images were reconstructed and checked for motion artefacts. Acquisition of each SPET image took approximately 120 minutes.

SPET Data Processing and Analysis

Images were reconstructed into five minute time periods. These were condensed into fifteen minute sequential time slots to improve the signal-to-noise. Reconstructed image data were assessed using Hermes software (Hermes Medical Solutions, Stockholm), a validated semi-automatic method of assessing tracer uptake in the basal ganglia[159]. Hermes was used to extract counts data for the left and right caudate, and

left and right putamen. The occipital cortex was used as a reference region since it contains negligible concentrations of D2 receptors[158].

Outcome Measures

Psychological assessments were administered at baseline (30 minutes prior to injection) and at 30, 80 and 120 minutes following the final injected (THC) pulse.

1. Psychotic Symptoms

Instrument: The PANSS, as in study 1.

2. *SPET data*

Counts in the caudate and putamen were compared to background (occipital cortex). The counts calculation used was (Area of Interest – Background) / Background. The subtraction index was then calculated: ((Area of Interest – Background) / Background)*100, as this addresses individual and scan differences[160]. The subtraction index (here-after referred to as the D2-binding Index) was used in statistical analyses.

3. Pharmocokinetics

Blood (5mL) was collected at baseline and at 1, 5, 15, 30, 60 and 120 minutes post-injection, and stored at -70ºC until analysis for [THC] and its major metabolites. Analysis were carried out as described in study 1.

Statistical analysis

Differences in PANSS scores under THC v placebo conditions were analysed using Friedman's test. Differences in the D2-binding index were analysed using a repeated

measures ANOVA. Factors were Treatment (THC v placebo), Region (left caudate, right caudate, left putamen, right putamen) and Time (0-45min bin, 75-120min bin). In addition, differences in the D2-Binding index [i.e. from 'baseline' (0-45min bin) *minus* post-treatment (75-120 min bin)] in the two treatment arms (THC v placebo) were compared using a paired t-test. Correlations between positive psychotic symptoms and changes in the D2-binding index were analysed using Spearman's rho. All analyses were two-tailed. Significance was accepted at $p<0.05$.

RESULTS

Of 11 recruited subjects, 10 completed both arms. Scanning failure in 1 session (a placebo arm) meant complete SPET data across both sessions was available for 9 subjects. Plasma concentrations over time following the administration of THC are shown in Figure 2.1.

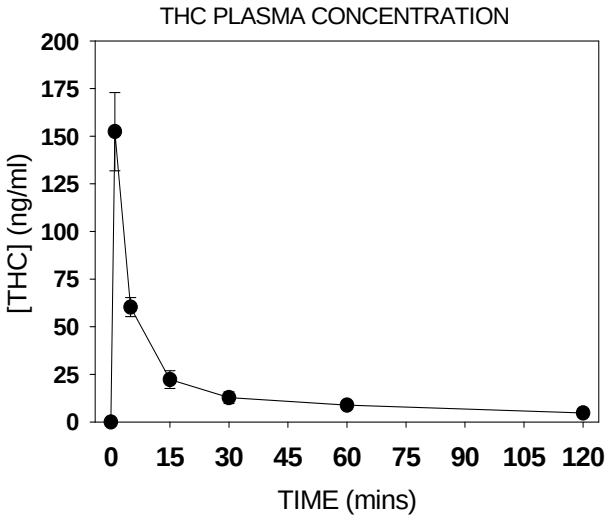


Figure 2.1 Mean plasma concentrations following the intravenous administration of THC (2.5mg). Error bars show ± 95% CI.

THC induced positive psychotic symptoms

Scores on the PANSS positive subscale were increased from baseline following THC but not placebo administration (Friedman's χ^2=28.1 p<0.001) (Figure 2.2).

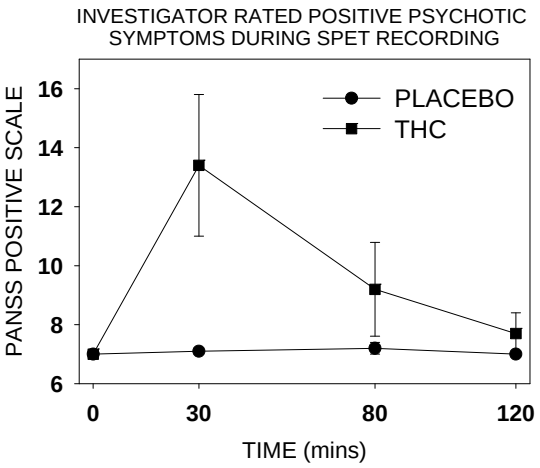


Figure 2.2 Following the administration of IV THC 2.5mg, healthy participants experienced positive psychotic symptoms (mean±s.e), as rated by The positive & negative syndrome scale (PANSS).

The D2 Binding Index ('Dopamine release')

There was a strong trend for increases in the D2 binding index in the 75-120min bin compared to the 0-45min bin (F=3.85, p=0.09), but no effect of treatment (F=1.3, p=0.3) and no interactions. In Figure 2.3, there is an impression of 'slower' IBZM accumulation in the caudate nuclei in the THC compared to the placebo treatment arm, but numerical differences were not significant; [even if the right and left caudate were averaged to reduce variance (t=0.95, p=0.4)].

Correlations

There was no relationship between PANSS positive symptom scores and change in the D2 binding index following THC treatment (rho=0.15, p=0.7)

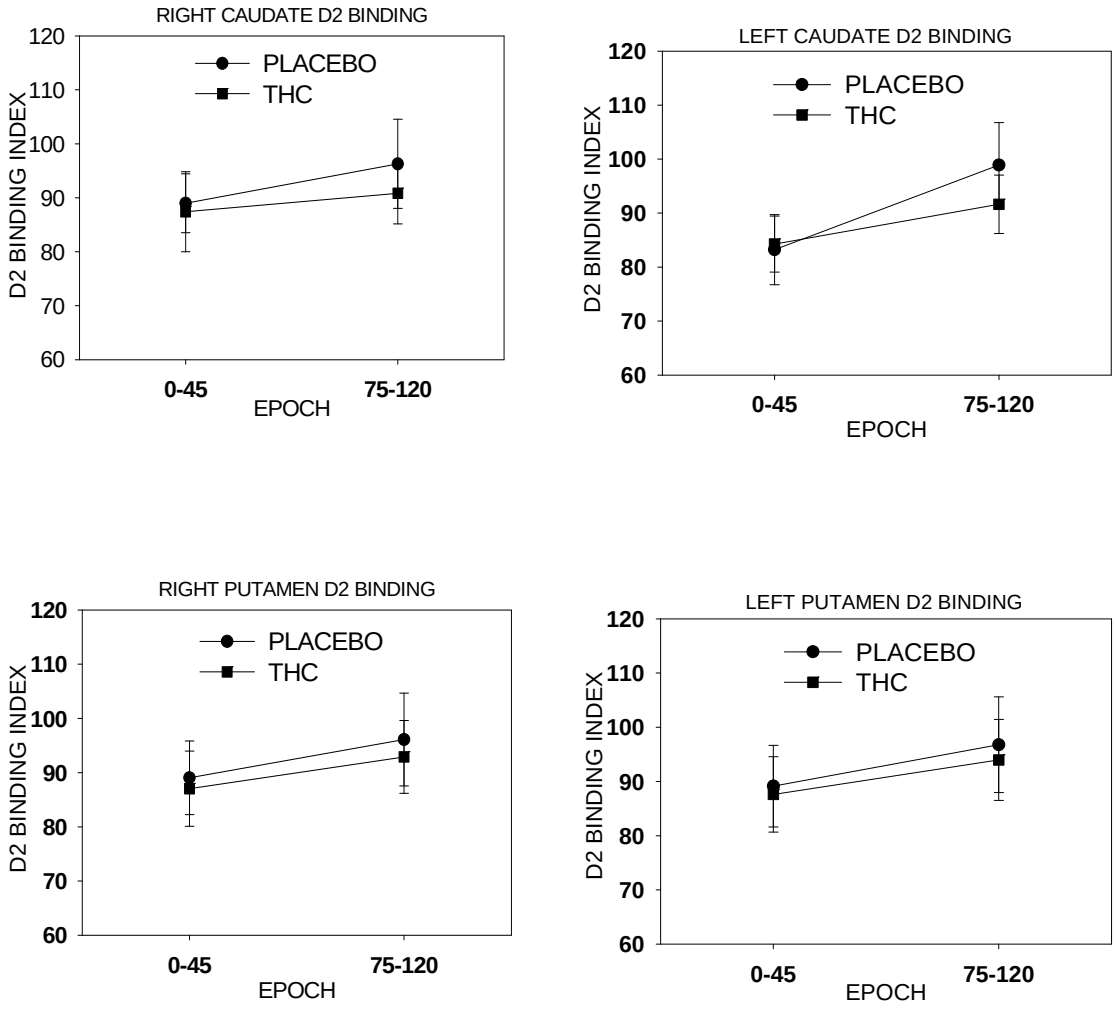


Fig 2.3 The D2 binding index (region of interest-background/background *100) in 4 basal ganglia regions (right & left, caudate nucleus & putamen), under THC versus placebo conditions. THC (2.5mg)/placebo were injected over 5-minutes, beginning at 45 minutes.

DISCUSSION

THC-psychosis and IBZM displacement (Dopamine release)

The main finding in this study is that IV-THC, at doses sufficient to elicit psychosis, shows no significant difference from placebo in stimulating dopamine release in the

striatum. This suggests that the psychotomimetic properties of THC are not mediated by activation of the mesostriatal dopamine system. Furthermore, in this [admittedly small] sample, positive psychotic symptoms and striatal dopamine release were not related. The most prominent THC-elicited psychopathology occurred in the domains of delusional ideation and suspiciousness. Other subjective experiences reported included thought echo (n=1), auditory hallucinations (n=2) and passivity phenomena (n=2).

Two recently published studies have used neurochemical imaging to measure dopamine release in man following THC. Utilising PET, Bossong and colleagues (2009) showed that specific [^{11}C]raclopride binding was decreased (by ~3.5%) in the ventral striatum and precommissural dorsal putamen after inhalation of THC, although THC-psychosis was not observed in any of the subjects (n=7). In a larger PET study (n=13), Stokes and co-workers (2009) found no significant difference in striatal [^{11}C] raclopride binding between oral THC and placebo sessions (although binding in the frontal and lateral temporal cortices was decreased under THC conditions[116]). In the Stokes et al study, participants reported perceptual illusions and rapidity of thinking. Overall, the three studies suggest that, relative to other substances; nicotine, cocaine and amphetamines (Laruelle et al., 2000) THC does not evoke large increments in striatal dopamine release as measured by neurochemical imaging in humans.

THC-psychosis and dopamine

The above interpretation is in keeping with two previous pharmacological studies. In stable, schizophrenic patients, the administration of IV THC (2.5mg and 5 mg) led to marked increases in PANSS-positive scores, despite ongoing D2-receptor based anti-

psychotic treatment[99]. Similarly, in healthy controls, pre-treatment with haloperidol had no effect on acute THC-psychosis[161], although see Liem Moolenaar et al 2010[162]. At present, there is very little evidence to support the hypothesis that THC-psychosis is mediated by excessively abnormal striatal DA release.

Limitations

The major limitation in the study here is that numbers are small, although similar in size to previous SPET/PET displacement studies assaying dopamine release following nicotine[163], amphetamines[164] or THC[165, 166]. Also SPET has poorer spatial resolution compared to PET. A strength is the use of IV-THC, which evokes a robust psychotic response, characterised by delusions of reference and suspiciousness. Future studies might take advantage of IV-THC and PET.

CONCLUSION

The basis for suspecting that dopamine mediates the pro-psychotic properties of THC is weak from the findings here, and other recent imaging studies.

CHAPTER 3

STUDY 3: The Effects of THC on brain oscillations

BACKGROUND

Animal work has shown begun to unravel the effects of THC on network dynamics. Robbe and colleagues showed that THC decreased the power of theta, gamma and ripple oscillations in the hippocampus, effects which could be blocked by CB_1 antagonists[58]. In agreement, Hajos and co-workers found that CB_1 agonists disrupted theta and gamma oscillations within the septo-hippocampal system, and disrupted the P50 auditory-gating response, one of the most robust intermediate phenotypes in schizophrenia[129].

In humans there have been several recent reports of the effects of cannabis on neuronal oscillations, recorded using electroencephalography (EEG). Gevins and colleagues analysed EEG power in 10 subjects, during the performance of cognitive tasks. Working and episodic memory performance was poorer following inhaled marijuana (3.5% THC). The principal EEG findings were decreased global theta power and reduced alpha band reactivity[167]. Similarly in a study of inhaled marijuana (0, 29, 49 & 69mg) in 16 participants, Bocker and co-workers observed a dose-dependent decrease of resting theta and beta power. Furthermore, decreases in theta power were related to slower WM performance[168].

AIMS

The starting point in the present study was to confirm the effect of THC on theta power. Thereafter the aim was to characterise the effects of THC on EEG coherence during WM performance. Coherence is a measure of the correlation between a pair of

signals as a function of frequency. It is regarded as an index of the functional relationship between two brain regions[124].

HYPOTHESIS

The most robust EEG changes under THC would be associated with THC-elicited psychopathology and cognitive impairment.

METHODS

Design

A randomised, double-blind placebo controlled crossover study in healthy volunteers of the effects of IV THC (1.25mg) on working-memory performance, psychopathology and concurrent EEG activity. [The lower dose was used to minimise participant drop-out rates].

Participants

Twenty healthy participants were recruited according to the criteria described in study 1, except that female participants were also recruited. (Female participants were also tested for possible pregnancy using a standard HCG urine screen). Sessions were performed at least two weeks apart and started between 0900 and 1400 h. Placebo and THC were administered under double-blind conditions, in a randomised counterbalanced order.

Pharmaceuticals

Dronabinol (THC) 1.25mg IV over 5-minutes, as in study 1.

Outcome Measures

Psychological assessments and self-rated scales were administered at baseline (30 minutes prior to injection) and at 30, 90 minutes post pharmaceutical.

1. Psychotic Symptoms

Instruments: The PANSS (see study 1).

2. Cognitive Testing

Immediately following the psychiatric assessment at 30-mins post-pharmaceutical, participants were administered a standard computerised version of the n-back task[149]. The n-back procedure has been used extensively to measure human working memory performance[149]. Participants were required to monitor a series of letters and report when the current letter matched the letter n integers back, where n=1 (1-back) or n=2 (2-back), the latter being more difficult. The task requires continuous updating of information stores. In contrast, in the 0-back condition (which does not require manipulation of material in WM), participants responded to the appearance of a pre-specified letter. Overall, the task consisted of alternating 30-second blocks of 0-back, 1-back and 2-back conditions, and lasted 6 minutes in total. Within blocks, letters were displayed every 2 seconds for 1 second. Written instructions were read out and participants were given a practice run to demonstrate their understanding of the rules. Subjects were seated ~66cm from a CRT monitor and instructed to report correct answers as rapidly as possible by pressing a joy-pad button with their R-index finger. Accuracy of responses and reaction-times were measured and stored digitally.

3. Pharmacokinetics

Blood samples were taken at baseline and at 1, 5, 15, 60, and 120 min after dosing, for analysis of [THC]. Analysis was carried out as per study 1.

4. Electroencephalography

All data recording and signal processing were performed in Neuroscan 4.3. EEG activity was recorded from 63 electrode sites using a Quik-Cap system (Compumedics), with a linked mastoid reference and ground at AFz. All impedances were maintained below 10 kO. Additional electrodes were placed at the outer canthi to measure horizontal electrooculographic (EOG) activity (monopolar with linked mastoid reference). Vertical EOG was measured using a bipolar recording with electrodes above and below the left eye. The EEG was sampled at 2000 Hz and corrected for eyeblinks using a regression approach. The corrected EEG was epoched, using a 10% Hanning window, into 2048 ms segments (-24 to 2024 ms with respect to each n-back letter stimulus). Epochs were baseline corrected. For each of the three n-back conditions, average power within the frequency bands delta (1–3.5Hz), theta (3.5–7 Hz), alpha (8–13 Hz), beta (14–25Hz), low-gamma (30–40 Hz), and high-gamma (60–70 Hz) bands was calculated using Fast Fourier Transform.

EEG power

For the power analysis, individual electrodes were grouped as left frontal, LF (F1, F3, F5, F7, AF3); right frontal, RF (F2, F4, F6, F8, AF4); left central, LC (C1, C3, FC1, FC3); right central, RC (C2, C4, FC2, FC4); left temporal, LT (FT7, T7, TP7, CP5, P7); right temporal, RT (FT8, T8, TP8, CP6, P8); left occipito-parietal, LOP (O1, PO5, PO3, P3, P1); and right occipito-parietal, ROP (O2, PO6, PO4, P4, P2). The

mean value from each group of electrodes was used for statistical analysis. The midline electrodes FZ, CZ, and PZ were analyzed individually.

EEG coherence

For the coherence analysis, the data were transformed to bipolar derivations. These derivations consist of pairs of neighboring electrodes at different scalp locations to eliminate the contribution of activity from a common reference to the coherence estimate. Bipolar channels were derived for left and right frontal and parietal regions (F3/F5; PO3/PO5; F4/F6; PO4/PO6). The measure of coherence used is equivalent to a Pearson's correlation performed with complex numbers. It measures the correlation (a value between 0 and 1) of EEG activity in a specific frequency band between two scalp locations. For each of the three n-back conditions, coherence measures were calculated between three prespecified inter-regions, left frontal–left parietal F3/F5-PO3/PO5, right frontal–right parietal F4/F6- PO4/PO6, and left frontal–right frontal F3/F5-F4/F6.

Statistical analyses

Distributions were checked for normality using Kolmogorov-Smirnov statistics. Non-parametric tests were used to analyse PANSS data, because of floor effects under placebo conditions; Thus differences between THC v placebo sessions were assessed using Friedman's test and relationships between PANSS scores and EEG measures were analysed using Spearman's rho. Accuracy and speed of performance in the n-back were analysed by repeated measures ANOVA, with task-difficulty and THC-treatment as within-subjects factors. Relationships between n-back data and EEG

measures were analysed by Pearson's correlation coefficient. Repeated measures ANOVA was used to analyse EEG power, with recording site, task-difficulty and THC-treatment as within-subjects factors. Individual ANOVAs were conducted for each frequency band delta *to* gamma. EEG coherence was analysed by repeated measures ANOVA with THC-treatment, region, task-difficulty and frequency as within-subjects factors. All ANOVAs were Bonferroni-corrected. Where sphericity assumptions were violated, Huynh-Feldt corrected statistics were used. Post-hoc t-tests were carried out where appropriate. Correlations between psychological outcomes and EEG measures were Bonferroni corrected to adjust for multiple comparisons. Otherwise significance was accepted at $p<0.05$. All analyses were 2-tailed.

RESULTS

Overall, 16 of 20 participants (7 male, 9 female) completed both sessions of the study. Two participants were lost to follow up. One subject discontinued her involvement and one subject experienced short-lived panic and the session was stopped prematurely. Mean age was 26 ± 6 years. Prior to experimental sessions, all urine drug screens were negative. Previous use of cannabis ranged from 2 to approximately 1000 occasions (median = 40). With regard to other drugs, 11 (of 16) had previously taken stimulants (cocaine/amphetamines), 6 had taken MDMA, 6 had taken psychedelics (psilocybin/LSD) and there was a single case each of previous ketamine

and GHB. Plasma concentrations of THC over the course over the experiment are summarised in Figure 3.1.

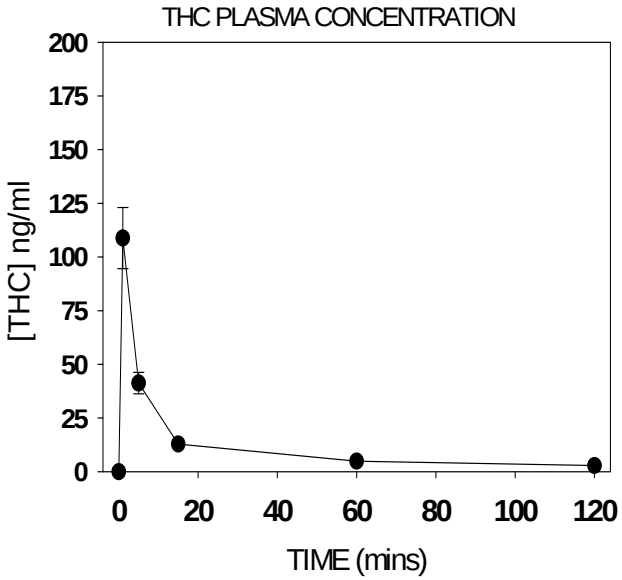


Figure 3.1 Mean plasma concentrations following the intravenous administration of THC (1.25mg). Error bars show ± 95% CI.

Psychopathology

Compared to placebo, THC increased positive (Friedman's χ^2=63.7, p<0.001) negative (Friedman's χ^2=56.0, p<0.001) and general PANSS scores (Friedman's χ^2=36.1, p<0.001). Increases were most pronounced at the 30-minute assessment point and tended back towards baseline by 90-minutes (Figure 2a, b). Overall, 40% of participants showed increases in PANSS positive symptom scores of >4 points at 30-minutes post-injection (Figure 3.2).

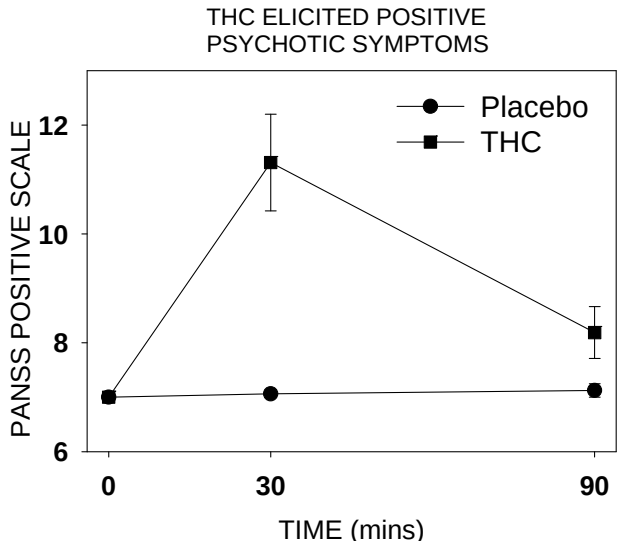
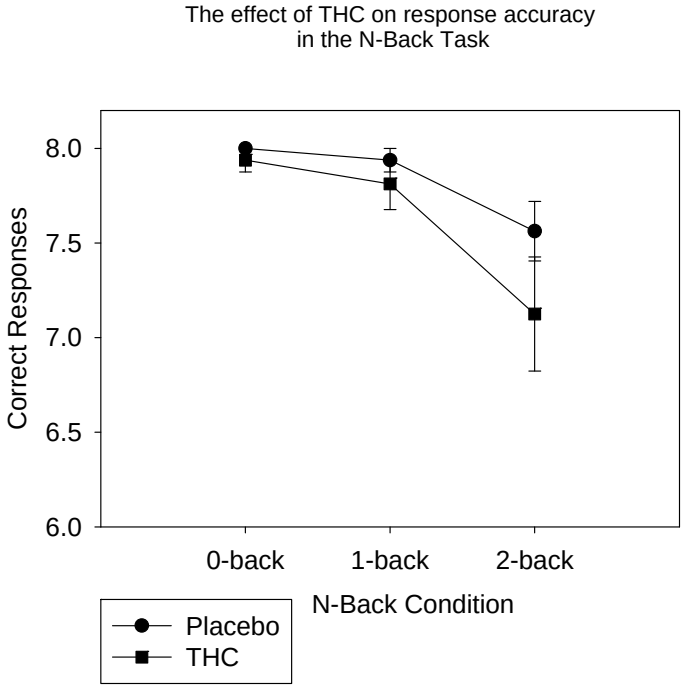


Figure 3.2 Following the administration of IV THC 1.25mg, healthy participants experienced positive psychotic symptoms (mean±s.e), as rated by the positive & negative syndrome scale (PANSS).

Cognitive performance

There was a trend towards reduced accuracy in the n-back task following treatment with THC ($F=2.95$, $p=0.11$). Accuracy was robustly affected by task-difficulty ($F=5.38$, $p<0.005$), with poorer performance in the 2-back condition compared to both the 1-back ($p<0.05$) and 0-back ($p<0.01$) conditions (Figure 3.3). In terms of accuracy, there was no THC-treatment x task-difficulty interaction.

Response times in the n-back task were slower under THC versus placebo conditions ($F=6.8$, $p<0.05$), and as task-difficulty increased ($F=32.3$, $p<0.001$). There was an interaction between THC-treatment x task-difficulty, in that the effect of THC was significantly greater as the n-back became more challenging ($F=7.74$, $p<0.005$) (Figure 3.3)



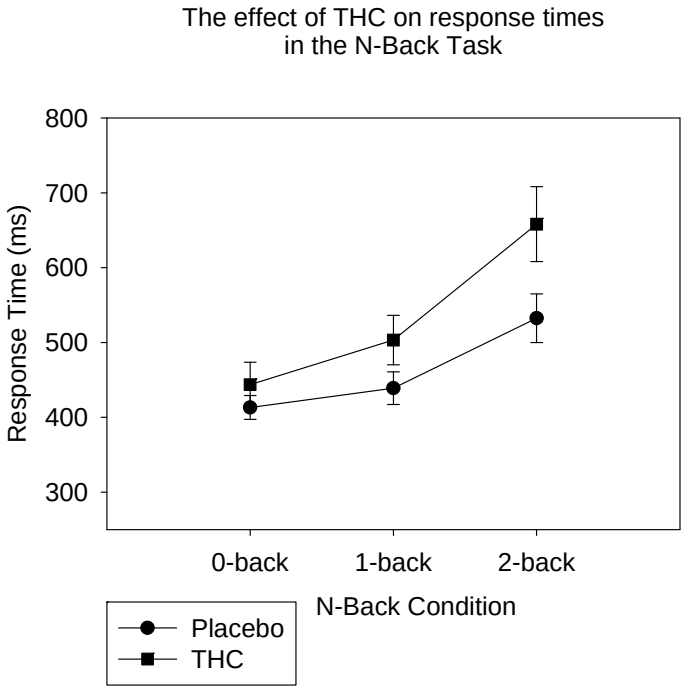


Figure 3.3 Under THC conditions, there was a trend for less accuracy in the most difficult condition of the N-Back task, but reaction times were significantly slower.

Electroencephalography

Power

THC decreased theta power ($F=23.5$, $p<0.001$),

of task difficulty or recording site (Figure 3.4 a, b, c). There was also a trend towards decreased alpha power under THC ($F=3.74$, $p=0.07$), with no treatment x task difficulty or treatment x recording site interactions. Power in the beta, delta and gamma bands was unaffected by THC.

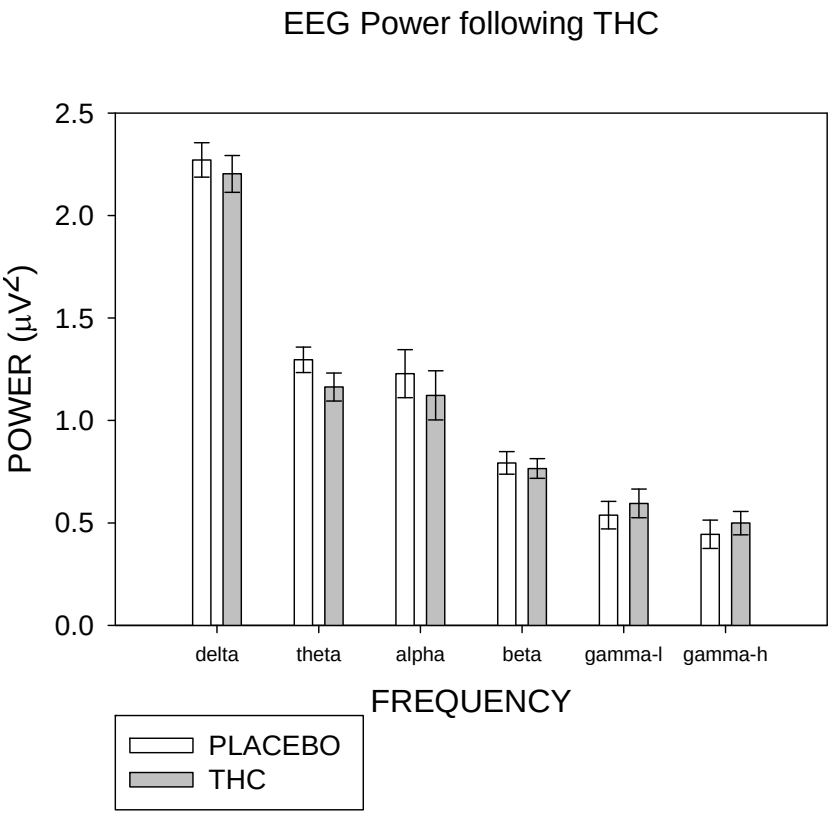


Figure 3.4a During working-memory performance, midline frontal theta power (electrode Fz) was reduced under THC conditions. There was a strong trend for a decrease in alpha power under THC, whereas delta, beta and gamma bands were unaffected.

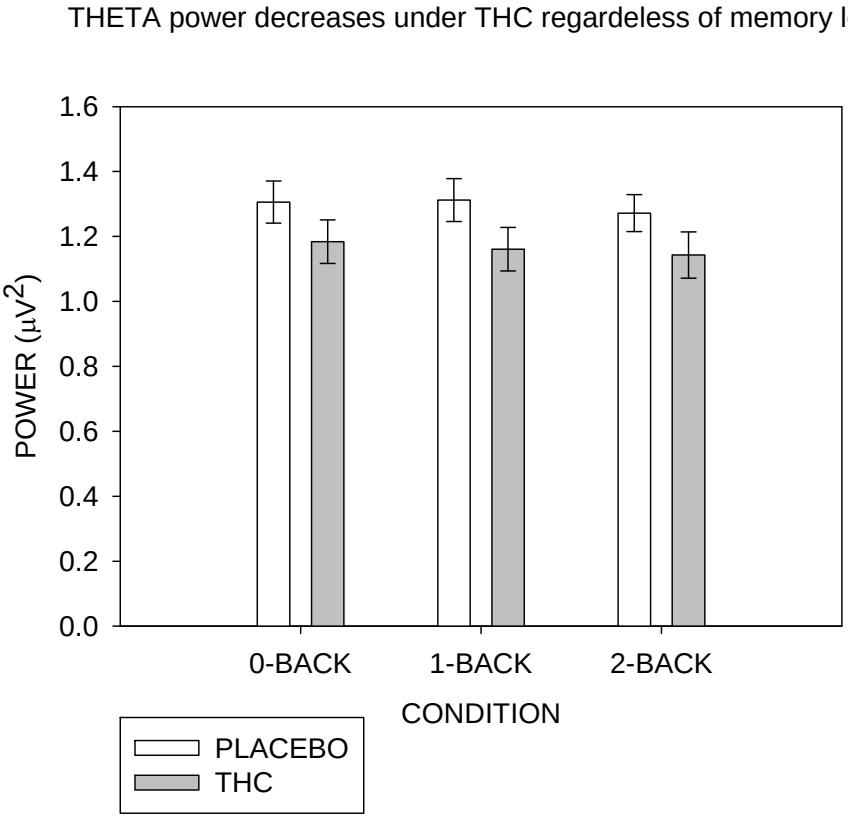


Figure 3.4b Under THC conditions, midline frontal theta power was reduced at all levels of task difficulty in the N-Back.

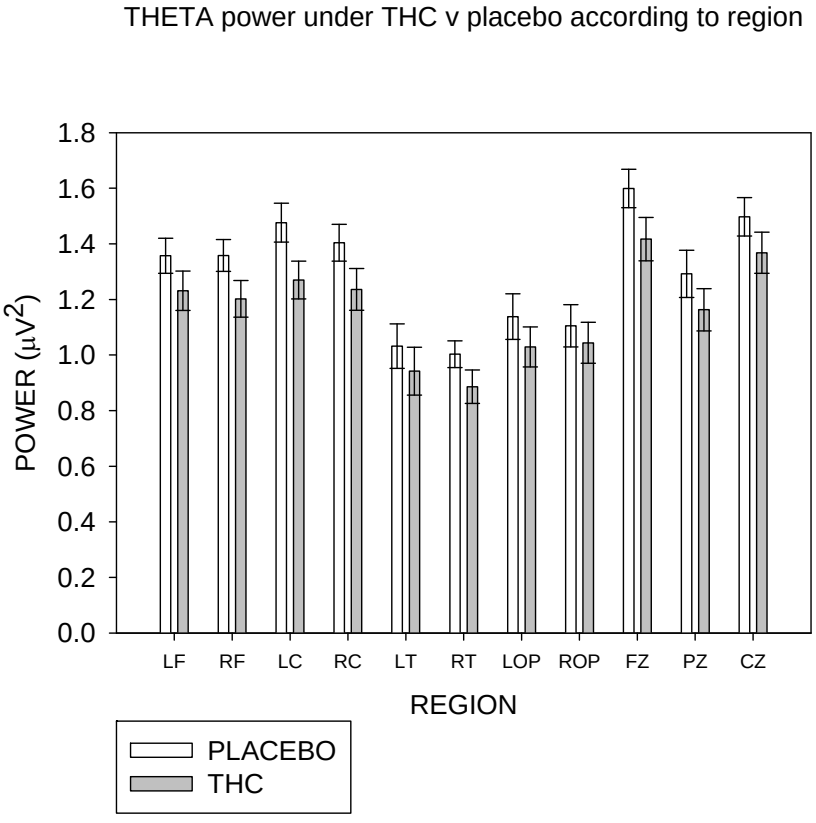


Figure 3.4c Under THC conditions, theta power was reduced across recording sites. (Left frontal, LF; Right Frontal, RF; Left central, LC; Right central, RC; Left temporal, LT; Right temporal, RT; Left occipitoparietal, LOP; Right occipitoparietal, ROP; Midline frontal, FZ; Midline parietal, PZ; Midline central, CZ.

EEG coherence

There were overall effects of region ($F=9.8$, $p<0.01$), frequency ($F=16.6$, $p<0.001$), task-difficulty ($F=24.3$, $p<0.001$), and THC ($F=6.1$, $p<0.05$). EEG coherence was greater between bi-frontal electrodes, compared to L-fronto-parietal ($p<0.05$) and R-fronto-parietal ($p<0.05$) electrode pairs. Compared to the 0-back condition, overall coherence increased under the 1-back ($p<0.001$) and 2-back conditions ($p<0.005$).

Interactive effects were region x frequency ($F=4.0$, $p=0.01$) and frequency x THC ($F=3.1$, $p<0.05$). There was a trend towards a 3-way interaction between region, frequency and THC-treatment ($F=2.0$, $p=0.09$).

Under placebo, bi-frontal coherence was largest in the theta band and theta coherence was greater between bi-frontal region compared to both the left ($p=0.01$) and right ($p<0.005$) fronto-parietal regions. THC selectively decreased coherence in the theta ($p<0.05$) and alpha ($p<0.05$) bands (Figure 3.5).

Correlations

EEG Power and psychopathology

Overall there was no correlation between *change-in* theta power, either globally or specifically at electrode Fz, and (1) reaction-time in the n-back task, (2) positive PANSS scores or (3) negative PANSS scores.

EEG coherence and psychopathology

I investigated possible relationships between reductions in theta and alpha coherence and three psychological outcomes, positive symptoms, negative symptoms and

reaction time in the n-back task. Since there were six comparisons in total, statistical significance was set at $p < 0.008$.

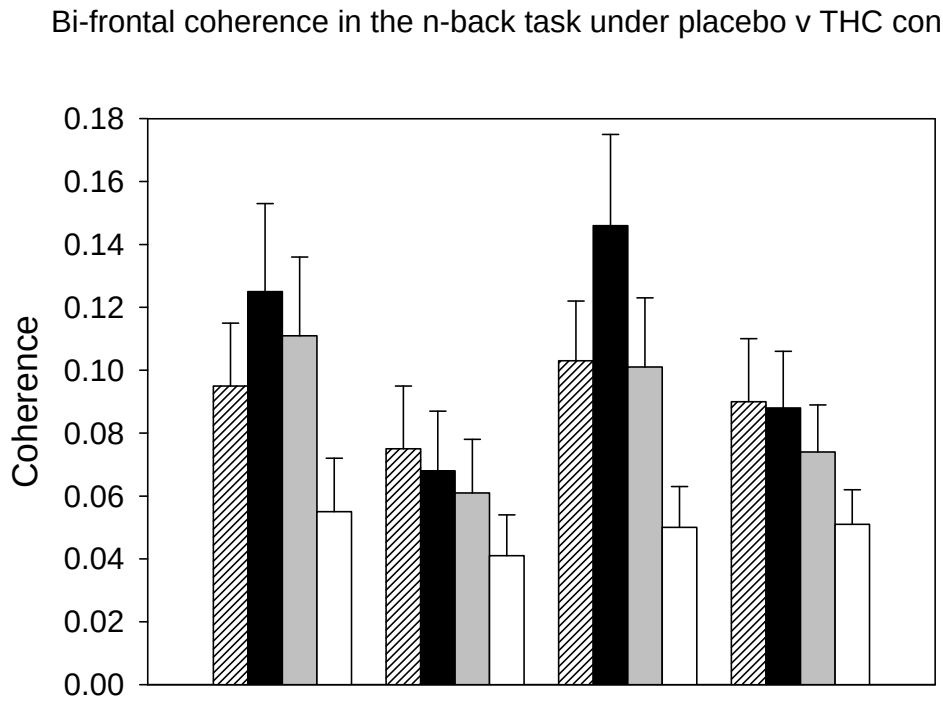


Figure 3.5 Coherence between left and right prefrontal regions, under THC v placebo, in the control (0-back) and most challenging (2-back) levels of the n-back task. Bars show coherence (mean±SEM) by frequency. (Diagonal stripes, delta; black, theta; grey, alpha; white, beta.) THC selectively decreased coherence in the theta ($p<0.05$) and alpha ($p<0.05$) bands.

The *change in* theta coherence (averaged over bi-frontal, and left and right fronto-parietal) under THC conditions was strongly associated with positive PANSS scores (rho=0.75, p=0.001), but neither negative symptoms nor reaction-time in the n-back. The relationship between theta coherence and positive symptoms was specific for the

bi-frontal region (rho=0.79, p<0.001). Change in left fronto-parietal or right fronto-parietal theta coherence was unrelated to positive symptoms. Reduced bi-frontal theta coherence occurred at all levels of the n-back, and survived the removal of 2 potential outliers (rho=0.69, p=0.006) (Figure 3.6).

There was a weaker relationship between the *change-in* alpha coherence and negative symptoms (Averaged rho=0.57, p=0.02) which was insufficiently robust to survive correction for multiple testing, and no relationship with positive symptoms nor reaction-time in the n-back.

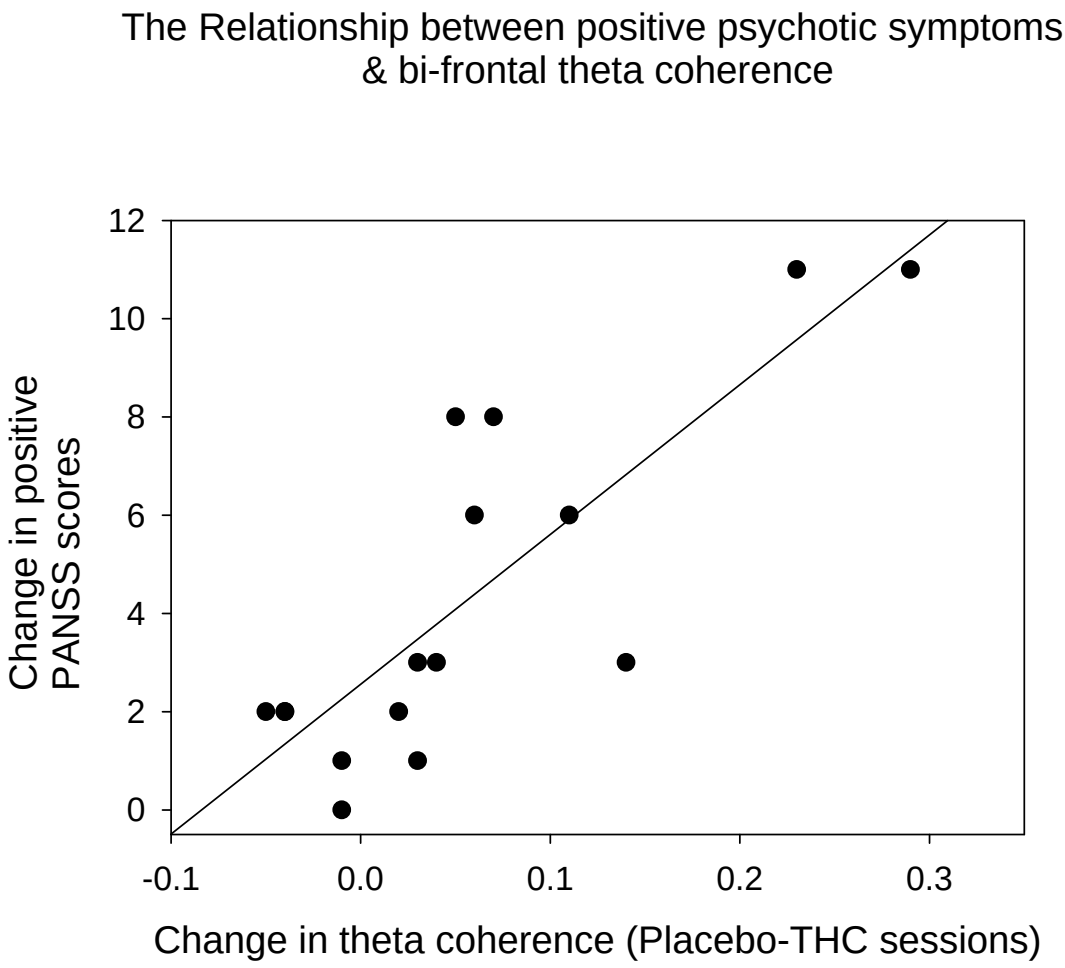


Figure 3.6 Reductions in theta coherence between left and right prefrontal regions under THC was correlated with positive psychotic symptoms (rho=0.79, p<0.001), and survived the removal of two potential outliers (rho=0.69, p=0.006).

DISCUSSION

The major finding from the present study was that THC decreased theta coherence between bi-frontal brain regions and that reductions from baseline were strongly associated with positive psychotic symptoms. Additional effects of THC in the current sample – transient psychosis, slower working-memory performance and global suppression of power in the theta band- are consistent with previous reports.

Previously there has been speculation that the pro-psychotic effects of THC stem from disruption of synchronised neural rhythms[129, 130]. The findings here provide experimental support.

CONCLUSION

Global theta power was reduced by THC – without any manifest psychopathological consequences. In contrast, there was a strong and specific association between THC-induced positive psychotic symptoms and reduced bi-frontal theta coherence. Impaired functional 'cross-talk' between the frontal lobes in the theta band might account for the pro-psychotic effects of THC/cannabis.

CHAPTER 4

STUDY 4: The effect of Cannabidiol on THC-psychosis (Pilot)

BACKGROUND

The pro-psychotic properties of cannabis are attributable to THC. Another plant-derived molecule, cannabidiol (CBD) is reported to display anti-psychotic properties in animal models and in humans[169-172]. Animal work and early human studies suggested that CBD could antagonise some of the pharmacological effects of THC[115, 173].

In the UK, new forms of cannabis (sinsemilla), which contain high concentrations of THC but negligible concentrations of CBD, now dominate the illicit cannabis market and there is concern that sinsemilla might be more hazardous for mental health than traditional cannabis[84]. Some have speculated that it is the absence of CBD, rather than rising concentrations of THC, which is important[82]. Here we tested whether CBD could inhibit the pro-psychotic effects of THC. [The receptor pharmacology of CBD is discussed in Section 7.2].

HYPOTHESIS

It was hypothesized that THC-elicited positive psychotic symptoms, would be inhibited by pre-treatment with CBD.

METHODS

Design

A within-subjects, placebo-controlled, double-blind investigation of whether intravenous (IV) CBD attenuates the acute psychotic reaction elicited by IV THC.

Participants

Six healthy participants were recruited according to the criteria described in study 3. Participants attended for 2 experimental sessions at least 2 weeks apart. Placebo and CBD were administered under double-blind conditions, in a randomised counterbalanced order, immediately prior to THC. All injections were administered over 5 minutes.

Pharmaceuticals

Dronabinol (THC) 1.25mg IV

Cannabidiol (CBD) 5mg IV (IV CBD/placebo was obtained from STI Pharmaceuticals UK).

Outcome Measures

Positive psychotic symptoms were assessed at baseline and at 30 minutes and 90-minutes post-THC using the PANSS rating scale (5-factor version).

Statistical Analyses

Differences in PANSS positive scores were analysed by the Mann-Whitney U test.

RESULTS

At 30 minutes post-dosing, THC-elicited PANSS positive symptoms were lower under CBD compared to placebo conditions ($z= -2.3$, $p<0.05$) (Fig 4.1).

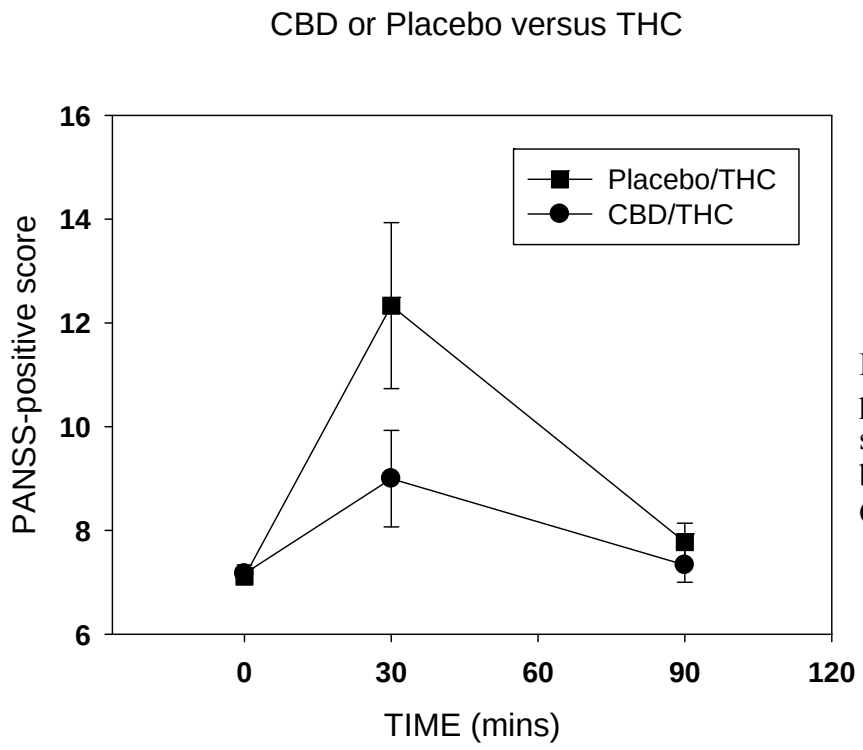


Figure 4.1 THC-elicited positive psychotic symptoms were decreased by pre-treatment with CBD.

DISCUSSION

The finding that 'a mixture' of CBD and THC is less psychotogenic than an equivalent dose of THC on its own suggests that CBD 'protects' against THC-induced psychosis. The results support the view that the psychotogenicity of sinsemilla may stem, in part, from an absence of CBD.

LIMITATIONS

Small sample size.

CHAPTER 5

STUDY 5: The effect of CBD on THC-psychosis and THC-elicited cognitive impairment

BACKGROUND

In study 4 it was found that that pre-treatment with IV CBD (5mg) inhibited IV THC (1.25mg) evoked positive psychotic symptoms, as measured by the Positive & Negative Syndrome Scale (PANSS), although the small sample size (crossover, n=6) limited definitive conclusions. Since then, a series of community studies have addressed this issue.

1. In a highly original design, Morgan and Curran measured trace cannabinoid levels in hair samples from regular cannabis users as well as scores of psychosis proneness as rated by the OLIFE (Oxford Liverpool Inventory of Life Experiences) instrument. Regular users who were grouped as THC-positive/CBD-negative scored higher than regular users who were positive for both cannabinoids on scores of unusual experiences[174].

2. In an epidemiological study in South London, Di Forti and colleagues compared patterns of drug use in people presenting with a first episode of psychosis with healthy controls. Patients and controls were equally likely to have ever taken cannabis and started at the same age. Although patients were more likely to be daily-users the most striking difference was that they were approximately 7-times more likely than controls to be users of sinsemilla[175].

3. In Holland, the most popular types of cannabis sold on the market are measured annually for THC and CBD content. Schubart and colleagues combined this information with data on cannabis use from approximately 1900 people, and found

that the THC/CBD ratio was related to subclinical psychotic experiences as rated by the CAPE-scale (Community Assessment of Psychic Experiences). Subjects who used products with a high THC/CBD ratio reported significantly higher CAPE-total scores than those using products with a low THC/CBD ratio. In heavy users, (spending upwards of 50-Euros/week on cannabis), higher CBD content was associated with lower scores on the CAPE-positive symptoms dimension[176].

HYPOTHESIS

Here I conducted a larger study (between groups, n=48) in which IV THC (1.5mg) followed pre-treatment with either oral CBD (600mg) or placebo. I hypothesized that, following IV THC, the group who had been pre-treated with CBD would show less positive symptoms and less cognitive impairment than the group that had been pre-treated with placebo.

METHODS

Design

A 2x3 mixed design. Participants were randomly allocated in a counterbalanced fashion to placebo or CBD groups. Placebo/CBD capsules were administered under double-blind conditions. Each participant was assessed in three separate sessions: 1. Baseline 2. Post-capsule 3. Post-THC. Participants underwent EEG, as in study 3.

Participants

A total of 48 participants were recruited according to the criteria in appendix 3.

Pharmaceuticals

Capsules (placebo/CBD) were administered 3h-30 minutes prior to IV THC challenge.

Pre-treatment; Cannabidiol (2 x 300mg capsules) or matching placebo. Oral capsules of CBD (300mg) and matching placebo were obtained from STI Pharmaceuticals UK. THC 1.25mg IV over 10 minutes.

Baseline Predictive Instruments

Prior to the experimental session, participants completed the following questionnaires online: The Green *et al* paranoid thoughts scale (GPTS) Part B, which provides a measure of trait paranoia[177], The Cannabis experiences questionnaire, which quantifies psychotic/dysphoric experiences following recreational cannabis use[178], and the Schizotypal personality questionnaire[179]. This permitted assessment of whether measures of 'psychosis-proneness' differed between the two groups.

Experimental Measures

Positive Psychotic Symptoms

The positive dimension was assessed using two instruments:

1. The PANSS, as in study 1.

2. The State-social-paranoia-scale (SSPS)

The State Social Paranoia Scale (SSPS)[180]

The SSPS has ten persecutory items (e.g. "Someone wanted me to feel threatened"), each rated on a 5-point scale, which conform to a recent definition of persecutory ideation. The SSPS has excellent internal reliability, adequate test-retest reliability, convergent validity with both independent interviewer ratings and self-report measures, and divergent validity with regard to measures of positive and neutral thinking[180].

Cognition

1. The Hopkins verbal learning Task-Revised (Verbal learning & memory)

The Hopkins Verbal Learning Task (HVLT-R)

In the HVLT-R, participants are tested in their immediate recall of 12 words (nouns from three taxonomic categories) after each of 3 learning trials. Here, delayed recall was assessed twenty minutes after the final learning trial. Three versions of the task were used, in the same order for each participant.

Statistical Analyses

Data were assessed for normality using Kolmogorov-Smirvov test statistics. In line with expectations, data on the PANSS and State-social-paranoia-scale were highly skewed necessitating the use of non-parametric approaches. Thus Friedmans test, a non-parametric repeated-measures test was used to analyse positive symptom scores on the PANSS and the State-social paranoia scale. In addition, for the PANSS we followed the approach of D'Souza and colleagues which is to categorize clinically significant psychosis as increases from baseline of >=3 points[99]. Thereafter the difference in the frequency of clinically significant THC-evoked psychotic reactions between the CBD and placebo groups was analysed using Pearson's Chi-square. Normally distributed data were analysed by a general linear model (GLM), specifically repeated-measures ANOVA. The Within-groups factor was SESSION (1. Baseline 2. Post-capsule 3. Post-THC). The between-groups factor was pre-treatment GROUP (1. CBD 2. Placebo). Post-hoc analyses were performed with Bonferroni correction. Relationships between psychosis scores and cognitive data were analysed using Spearman's rank correlation coefficient. Significance was accepted at p values <0.05. All comparisons were two-tailed.

RESULTS

Forty-eight subjects completed the experimental protocol (Placebo-group n=26; CBD-group n=22). In three subjects, failure of cannulation prevented the administration of THC, and data acquired up to that point was not used in any of the analyses. The two groups were adequately matched for demographic variables, baseline measures of 'psychosis-proneness' and previous drug use (Table 5.1).

Variable	**PLACEBO-group**	**CBD group**	***p***
Age (years)	26 (±4)	25 (±3)	ns
Sex ratio (m:f)	14:12	13:9	ns
BMI	25 (±5)	25 (±4)	ns
SPQ (Total)	11.1 (±7.0)	12.1 (±11.2)	ns
CEQ (Paranoia/dysphoria)	43.0 (±9.1)	42.8 (±10.4)	ns
The Green Paranoia scale	19.3 (±5.0)	23.7 (±10.2)	0.08
Previous cannabis use (Episodes)	118 (±218)	137 (±234)	ns
Age at first cannabis use	16 (±2)	17 (±2)	ns
Previous Drug Use (Yes)			
'Ecstasy'	62.5%	48%	ns
Cocaine	54%	40%	ns
'Acid'	21%	20%	ns
Ketamine	21%	32%	ns
Amphetamines	13%	16%	ns
Mephedrone	17%	36%	ns

Table 5.1 Sample characteristics at baseline. The 2 groups (CBD & PLACEBO) were adequately matched for demographic variables, 'psychosis-proneness' as indexed by the SPQ, CEQ and Green et al Paranoia Scale, and for previous illicit drug use. BMI Basal metabolic index; SPQ Schizotypal Personality Questionnaire; CEQ Cannabis Experiences Questionnaire.

Pharmacokinetics

The plasma concentrations of CBD and THC over time are shown in Figure 5.1. Plasma concentrations of CBD peaked at the 3h 45mins time-point, before beginning

to decrease. THC concentrations were not significantly different between the group pre-treated with CBD and the group pre-treated with placebo at 5-minutes (p=0.5), 30-minutes (p=0.5) and 80-minutes (p=0.6) post-THC administration. Plasma concentrations (mean ng/ml±SEM) of the psychoactive metabolite 11-OH THC were higher in the CBD group compared to the placebo group at the 5-minute (2.13±0.43 v 0.37±0.13, p=0.001), the 30-minute (1.18±0.32 v 0.00±0.00, p<0.005) and 80-minute (0.69±0.19 v 0.00±0.00, p<0.005).

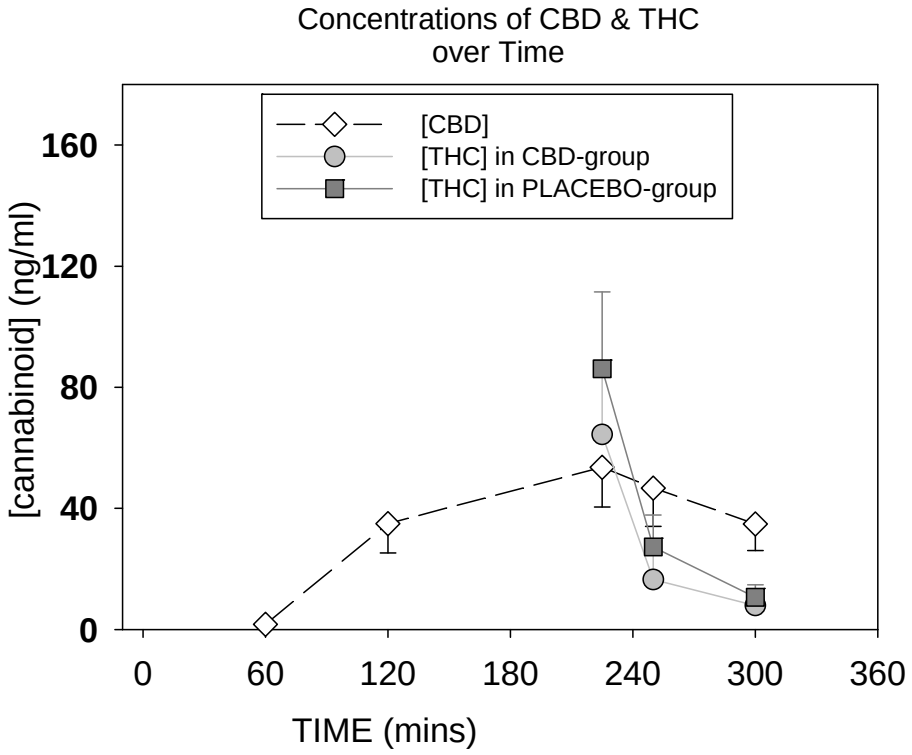


Figure 5.1 Plasma cannabinoid concentrations (mean ±SEM). Oral CBD (600mg) was administered at 0-minutes. THC (1.5mg) was administered by slow IV injection from 210-220 minutes. In the CBD pre-treated group and the placebo pre-treated group, differences in plasma THC concentrations at three successive sampling points, were not statistically significant. With respect to THC administration, plasma [THC] was assayed at 5, 30 and 80 minutes post-injection.

Positive psychotic symptoms

PANSS-positive Scores

Compared to baseline conditions, THC administration increased PANSS positive scores, regardless of whether pre-treatment was with CBD (χ^2=19.5, p<0.000) or placebo (χ^2=26.0, p<0.000). However clinically-significant positive symptoms following THC, defined as an increase in PANSS positive scores of >=3 points, were more common in the group pre-treated with placebo (11 of 26 cases) compared to the group pre-treated with CBD (3 of 22 cases), (χ^2=4.74, p<0.05) (Table 5.2).

State-Social-Paranoia-Scale Scores

Pre-treatment with CBD inhibited THC-elicited paranoia as measured by the State-social-paranoia-scale. Following the administration of THC, paranoia scores increased in the group pre-treated with placebo (χ^2=16.0, p<0.000), whereas there was no change in paranoia scores from baseline in the group pre-treated with CBD (χ^2=2.0, p=0.37) (Figure 5.2).

The Hopkins Verbal Learning Task

Immediate Recall

Immediate recall was poorer following THC, regardless of group (CBD-group F=10.5, p<0.005; placebo-group F=12.6, p<0.000). Post-hoc analysis revealed differences between post-THC and baseline performance, significantly in the placebo-group (p<0.005), and at the level of a strong trend in the CBD-group (p=0.06). Differences between post-THC and post-capsule performance were significant in the CBD-group (p<0.000) and the placebo group (p<0.005).

		PRE-TREATMENT	
THC-PSYCHOSIS		PLACEBO-group	CBD-group
No	Count	**15**	**19**
	Expected Count	18.4	15.6
Yes	Count	**11**	**3**
	Expected Count	7.6	6.4
Pearson Chi-Square=4.74, $p<0.05$ (0 cells have expected count less than 5)			
Event rate (psychosis)		42%	14%
Odds of psychosis		0.73	0.16
Absolute risk reduction		28%	
Relative Risk		0.33	
Relative risk reduction		67%	
Odds ratio		**0.22**	

Table 5.2 Pre-treatment with Cannabidiol, CBD (600mg po) reduced the odds of developing an acute psychotic reaction to delta-9-tetrahydrocannabinol, THC (1.5mg IV). Psychosis was defined as a >= 3-point increase from baseline on the PANSS positive subscale.

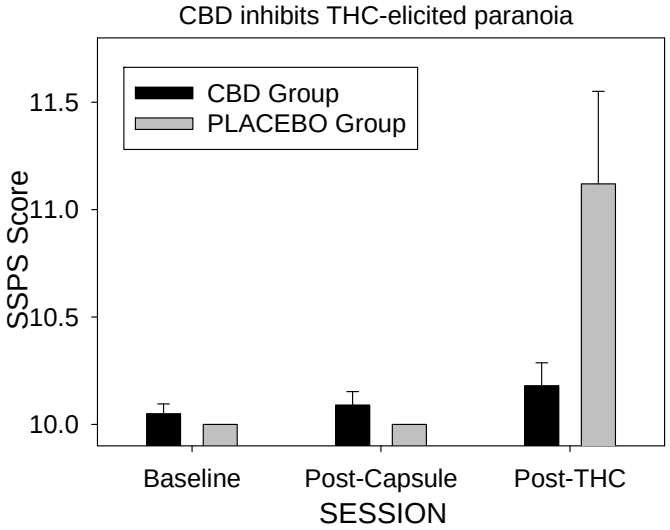


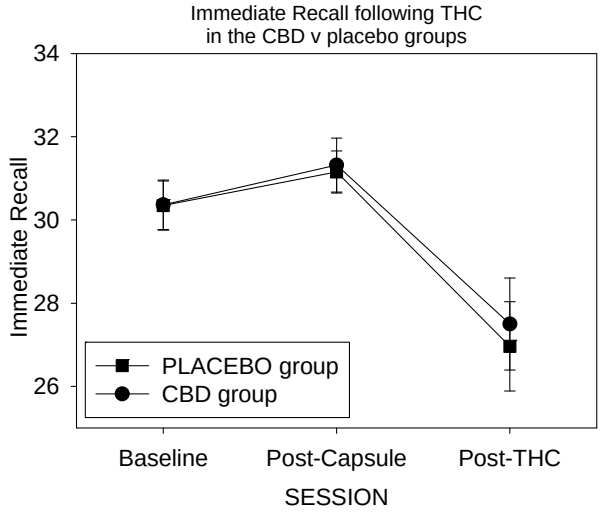
Figure 5.2 Pre-treatment with Cannabidiol, CBD (600mg po) inhibited delta-9-tetrahydrocannabinol, THC (1.5mg IV) evoked paranoia, as measured by The State-social-paranoia-scale (SSPS), (mean ±SEM).

Following THC, immediate recall was 2.9 (±5.3) and 3.6 (±4.5) items fewer in the CBD and placebo groups respectively, compared to baseline, a non-significant between-groups difference (p=0.6), (Figure 5.3a)

Delayed Recall

Delayed recall was poorer following THC in the placebo-group ($F=7.7$, $p<0.01$) but not in the CBD-group ($F=1.5$, $p=0.2$). Post-hoc analysis in the placebo-group revealed differences between post-THC and baseline ($p<0.05$) and between post-THC and post-capsule performance ($p<0.05$). Corresponding analyses in the CBD-group were $p=1.0$ and $p=0.6$ respectively. Following THC, delayed recall decreased from baseline by 10.7% (±18.9%) in the placebo group and by 0.4% (±9.7) in the CBD-group, a significant between-groups difference ($t=2.39$, $p<0.05$), (Figure 5.3b)

***A-posteriori*,** we explored if there were relationships between impaired delayed recall and positive psychotic symptoms, post-THC. In the placebo group, poorer delayed recall was related to the magnitude of PANSS-positive symptoms, at the level of a strong trend (Spearman's rho=0.3, $p=0.09$). The relationship between poorer delayed recall and higher scores on the state-social-paranoia scale was stronger and reached significance (Spearman's rho=0.5, $p<0.05$).



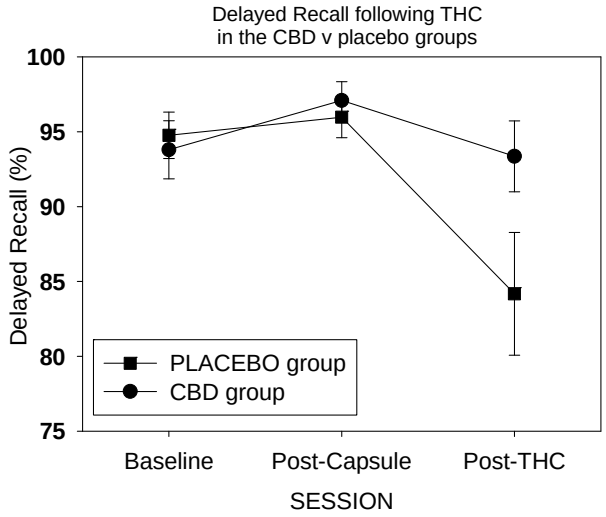


Figure 5.3 (a) Immediate recall in the HVLT-R (mean ±SEM) was poorer following IV THC (1.5mg), in both the placebo and CBD (600mg po) pre-treated groups. (b) Delayed Recall was poorer following THC in the placebo but not the CBD pre-treated group. HVLT-R, The Hopkins Verbal Learning Task-revised.

DISCUSSION

The major findings here are that pre-treatment with CBD decreased THC-elicited psychosis and inhibited the detrimental effects of THC on episodic memory.

Cannabinoids and Psychosis

The majority of community-based studies that have addressed the issue have proposed that cannabis products lacking CBD are more psychotogenic than products that contain CBD[174-176], but see Morgan *et al*, 2010[181]. The findings in the present study provide strong support for this idea. Here, on the PANSS (an investigator-rated scale), clinically significant THC-psychosis was less likely under CBD *versus* placebo conditions. On the SSPS (a participant rated scale) THC-elicited paranoid thinking was inhibited under CBD conditions.

Cannabinoids and Memory

Cognitive performance was poorer following THC specifically in the domains of working and episodic-memory, which is in keeping with previous reports (reviewed in Ranganathan and D'Souza, 2006[154]; Solowij and Michie, 2007[64]). Here, pre-treatment with CBD 'protected' episodic-memory from the impact of THC, whereas working-memory remained 'vulnerable' to a similar degree.

This result is in broad agreement with a community-based study carried out in London by Morgan and Curran: Volunteers were assessed at home under the influence of their own chosen brand of cannabis, a sample of which was subsequently tested for THC and CBD content. It was found that higher levels of CBD in "street cannabis" appeared to protect against impairments in immediate and delayed prose recall [181].

Systems Pharmacology

How THC impacts upon episodic memory is reasonably well understood. Episodic memory depends upon the integrity of the hippocampal circuitry. Numerous animal studies have shown that CB_1 agonists disrupt processes within the hippocampus that are believed to be at the heart of learning and memory - network oscillations, neuronal synchrony and plasticity[182-185]. Recently, CB_1 agonists have become a useful tool in hippocampal research. This is because CB_1 agonists disrupt synchronicity, without altering the firing rates of individual neurons in the network – a unique property amongst drugs which impact upon hippocampal function[58].

The mechanisms underlying the pro-psychotic properties of THC are less well understood. It has been suggested that disrupted network oscillations are important [129, 130]. The findings in study four provide experimental support.

In the present data set, there was a relationship between THC-psychosis and THC-elicited impairments in episodic memory. This was also observed in Study 1.

Pharmacokinetics

It is feasible that the differences between the CBD group and the placebo group emerged, not from a pharmacodynamic effect, but from an effect of CBD on the metabolism of THC. CBD is known to inhibit the metabolism of THC in rodents [increasing the plasma concentration of THC and decreasing the plasma concentration of the psychoactive metabolite 11-OH THC][186-189] although the effect is dependent on the time interval between administration of the 2 drugs[190]. To date, there is a paucity

of data on the pharmacokinetics of CBD/THC in humans. Here it was found that there was no difference between THC plasma concentrations in the CBD pre-treated versus the placebo group. At odds with the rodent studies described above, 11-OH THC concentrations were significantly higher in the CBD versus the placebo pre-treted group. The reason for this is unknown and clearly further studies in humans are required to address this issue.

Strengths and Limitations

In laboratory-based, pharmacological studies pure, synthetic preparations can be administered, at a set dose. This is particularly relevant for cannabinoid studies, because 'street cannabis' contains a multitude of other molecules, some of which are known to be pharmacologically active. One example is Δ^9- Tetrahydrocannabivarin (THCV), a CB_1 receptor antagonist at low doses, an agonist at higher doses[81]. A limitation in the present study is that only one dose of CBD was investigated. Future studies might examine if higher CBD doses, or indeed extended dosing over several days produces stronger 'protective effects' or if protection extends to additional domains such as working-memory.

CONCLUSONS

Previous epidemiological and experimental studies have suggested that cannabis-products lacking CBD are more psychotogenic than products containing CBD. The findings here provide strong support. Under controlled experimental conditions, CBD decreased THC-elicited positive psychotic symptoms and 'protected' hippocampal-dependent memory from the impact of THC.

CHAPTER 6

SUMMARY

1. In a proportion of subjects, THC elicited positive psychotic symptoms, which appeared to be distinct from anxiety.

THC also elicited negative symptoms, which appeared to be distinct form sedation.

However the most common effect of THC is disruption in the sense of passage of time, attention and concentration (Appendix 1).

2. In general THC impaired working memory, episodic memory and executive function. Impairments in episodic memory were related to positive psychotic symptoms in study 1, and this was replicated in study 5.

3. There was little evidence for an effect of THC on striatal dopamine release, despite the induction of positive psychotic symptoms (including 1st rank symptoms). This is in agreement with two recent imaging studies.

4. Under THC conditions, EEG power in the theta band was decreased. This is in keeping with animal work and two previous EEG studies. However decreased theta power did not show any relationships with the psychological manifestations of THC.

Under THC conditions, theta coherence between the two frontal lobes was diminished. The decrease in bi-frontal theta coherence relative to baseline was strongly correlated with the magnitude of positive psychotic symptoms.

5. Preliminary findings that CBD inhibits THC-elicited psychosis (Study 4) were confirmed in a larger study (Study 5). Additionally CBD inhibited the detrimental

effect of THC on episodic memory. The findings are in agreement with recent community-based studies that suggest that cannabis products lacking CBD are more hazardous for psychological health.

CHAPTER 7

DISCUSSION

THC psychosis

There are four main drug-models of endogenous psychosis: LSD, amphetamine, ketamine and THC. What is common between the different classes of drug is the promotion of a fundamental change in the subject's experience of reality, whether acutely during drug intoxication or as a result of an adaptive process secondary to repeated use. Comparing the different drug-models, CB_1 agonists can elicit a psychotic reaction in otherwise healthy controls after a single exposure, in contrast to stimulants (amphetamine/cocaine) where repeated use is a pre-requisite (A2.8). And repeated use of cannabis (especially forms high in THC-content) is a risk factor for the genesis of schizophrenia[28, 175], in contrast to ketamine where no association has as yet been found.

Is dopamine involved in THC psychosis?

The glutamate and GABA inputs to the band of dopamine neurons in the ventral midbrain express CB1 receptors and numerous animal studies have shown that exogenous cannabinoids alter the balance of excitation and inhibition which 'reach' dopamine cells[47, 155]. Although a net inhibition of dopamine cells has been described[191], by far the most commonly documented effect is an increase in firing and elevations of dopamine release in the striatum[155] .

Hence there are theoretical grounds for the hypothesis that the pro-psychotic effects of THC arise via dopamine. However the evidence to date has not been supportive. In

study 2 here, and in two additional neuroimaging studies[165, 166], there was no evidence for *substantial* dopamine release in the striatum following THC.

Abnormal Oscillations & THC psychosis

Previously there has been speculation that the pro-psychotic effects of THC stem from disruption of synchronised neural rhythms[129, 130]. The major finding here provides experimental support. It may be considered that THC elicits a 'lesion', at the molecular level which can 'push' an otherwise healthy nervous system towards acute psychosis, hastens the onset of psychotic-breakdown in those destined to develop schizophrenia and provokes acute relapse in established cases[192]. The precise nature (and location) of the molecular lesion, downstream of CB_1 receptors is unknown. But the most likely scenario is that THC disrupts the intricacies of fast amino-acid based neurotransmission; and in doing so, disrupts network oscillations which depend, in-part, upon reciprocal glutamate and GABA-ergic connections[58, 129, 193-195].

The findings in study 3 point to the pre-frontal cortex and implicate the theta band. Reduced bi-frontal (but not fronto-parietal) coherence from baseline was strongly associated with positive psychotic symptoms. This was not the case for theta power. The simplest interpretation is that acute THC-psychosis is associated with disruption in long-distance synchrony but not with disruption of local theta rhythms. This interpretation is broadly in keeping with the disconnection hypothesis, in which impaired functional connectivity between brain regions underlies schizophrenic symptoms[196] Some caution is required in attributing the pro-psychotic effects of THC to a direct action within the frontal cortices. It is likely that theta oscillations within limbic regions were also disrupted by THC[58, 129]. Thus an apparent cortical lesion

might *only* be a marker for a cortico-limbic lesion which is 'closer' to the pathophysiology of THC. For example Df(16)$A^{+/-}$ mice, (which mimic one of the largest genetic risk-factors for schizophrenia, a microdeletion on human chromosome 22q11.2), show reduced phase-locking of pre-frontal cells to hippocampal theta rhythms and reduced coherence of pre-frontal and hippocampal local field potentials[197].

Abnormal oscillations & endogenous psychosis

Abnormal neural oscillations occur in schizophrenic patients, and their relatives[194, 195]. The list of mental faculties affected by schizophrenia: perception, learning & memory, will, thinking; are also those which depend upon synchronised neuronal rhythms[195]. Initial work focussed on the gamma band, given the role of gamma-synchrony in Gestalt perception; the binding of separate sensory elements into a perceptual whole[198]. Numerous studies have now shown abnormal gamma rhythms in schizophrenia, and a feasible histological correlate – neurochemical deficiencies in fast-spiking GABA-ergic interneurons[125]. But it has become clear that abnormalities are not confined to gamma rhythms[194, 195]. In the context of the findings reported here, studies in schizophrenic patients have reported decreased power of frontal theta oscillations during performance of WM tasks[199, 200]. Ford and colleagues compared the EEG of speech production versus listening, in patients and controls. Speech production was associated with increased coherence across classical left hemisphere language regions within the theta band, in healthy controls but not in schizophrenic patients[201]. The authors concluded that reduced fronto-temporal functional connectivity in schizophrenia could lead to the attribution of self-generated speech to an external source.

Theta waves

Although theta oscillations have been implicated in the processes of working and episodic memory, their role is likely to be more general. Nowhere is this more apparent than in studies of *septo*-hippocampal theta in rodents, stretching back over sixty years. The list of putative behavioural correlates is extensive, and has been the source of some controversy, but "volition" or "will" is believed to play a critical role in theta generation[137].

At present the relationship between hippocampal theta and scalp recorded theta remains a matter of debate. Intra-cranial recording in humans (iEEG) indicate that there are 'independent' local cortical generators of theta[202]. In contrast, others have speculated that the cortex and hippocampal formation might operate as an integrated unit by means of synchronisation in the theta band[203]. In support of this idea, recent animal work has shown that neocortical spikes can be phase-locked to hippocampal theta oscillations[204]. Similarly, coupling of neocortical and hippocampal theta rhythms has been observed[205].

Despite the unresolved issues, there is a consensus that theta oscillations are important for long-distance 'cross-talk' between brain regions[137, 195, 198]. It is also appreciated that faster oscillations can 'nest' within slower oscillations; thus the power of gamma rhythms rises and falls on a slower theta wave[206].

7.2 Cannabidiol: Potential Utility

Studies 4 and 5 indicate that CBD can inhibit the pro-psychotic effects of THC. This is in keeping with findings from community-based studies[174-176]. Overall this has important public-health implications. The laboratory studies carried out here can be thought of as modelling acute cannabis exposure, whereas community studies represent repeated exposure. Both approaches point in the same direction: the absence of CBD is associated with increased THC-elicited psychosis and cognitive impairment.

There is also interest in whether CBD represents a useful medicine for psychiatry. In standard animal models, CBD has an anti-psychotic signature. Efficacy has been demonstrated in the apomorphine, amphetamine, ketamine, conditioned avoidance and pre-pulse-inhibition (PPI) models[72, 77, 78, 207]. CBD displays efficacy without eliciting motor side-effects (catalepsy)[72]. Preliminary findings suggest that CBD inhibits L-DOPA elicited psychosis in Parkinson's disease (Zuardi et al 2009 *J Psychoparmacol* **23**). Also, in a 4-week RCT in acute schizophrenic patients (n=42), CBD had equal efficacy with amisulpride, and superior tolerability[80].

Receptor Pharmacology

CBD targets a number of receptor, re-uptake and enzymatic. Deciphering the specific mechanism which lies at the root of a particular pharmacological property is challenging, particularly for CNS effects: At low concentrations CBD antagonises the GPR55 receptor[208]. There is evidence that GPR55 receptors are localised at glutamate terminals, where they function to amplify pre-synaptic glutamate release (Ross, personal communication). Blockade of GPR55, and inhibition of excessive glutamate release could feasibly underlie the anti-psychotic (and the anti-convulsant/ neuroprotective) properties of CBD. Similarly, at low concentration CBD antagonises

tissue responses to CB_1 agonists (despite low affinity for the orthosteric site of the CB_1 receptor)[81]. Antagonism of CB_1 signalling is the most parsimonious explanation for the ability of CBD to inhibit THC elicited psychosis. At low concentration, CBD inhibits adenosine re-uptake, which may account for some of the observed behavioural effects. At higher concentrations, CBD is an agonist as the 5-HT_{1A} receptor, a mechanism which has been proposed to underlie its *acute* anxiolytic properties [although 5-HT_{1A} mediated anxiolysis typically requires chronic dosing]. . Similarly at higher concentrations, CBD inhibits the cellular uptake and metabolism of anandamide. Some authorities have proposed the latter mechanism as important for the anti-psychotic effect of CBD[80].

Future Work

CBD

The focus now is on whether CBD constitutes a useful medicine in psychiatric patients. Preliminary work suggests that CBD has utility in psychotic illness. In particular, CBD may be an attractive candidate for the at-risk-mental-state (the pro-dromal phase of psychotic illness), particularly because of minimal side effects and good tolerability.

Oscillations

The effect of THC on bi-frontal theta coherence and the relationship with positive psychotic symptoms requires replication. Also, it is hypothesized that CBD inhibits the EEG effects of THC.

APPENDIX I

The following questionnaire was devised on the basis of verbal reports from the participants in studies one and two (Table A1.1). The scale was used in subsequent experiments. Figures A1.1-A1.3 show endorsement of items at IV THC (1.25, 1.5 and 2.5mg) and THC 1.5mg / CBD 600mg p.o.

ITEM	No	Minimal	Moderate	Strong	Extreme
1. Currently I'm interested in objects in the environment, more than usual					
2. My thinking is much faster than usual					
3. This experience is frightening.					
4. This feels like a set up.					
5. My perception of time is altered.					
6. I feel sleepy					
7. My thoughts are more special or significant than usual.					
8. My thoughts or movements seem to have a life of their own					
9. I'm worried for my mental or physical health					
10. I'm paranoid about the researchers					
11. My perception of objects is altered.					
12. I feel agitated					
13. I'm experiencing profound insights					
14. There is an unusual delay between my thinking and speaking.					
15. I'm worried this state of mind won't end.					
16. I believe I'm being made a fool of.					
17. Sounds are distorted.					
18. I can't focus my attention					
19. I can't sustain my concentration					
20. People are are saying things with 'hidden' or double meanings					
21. I am unsure if I have just been thinking a thing or have actually said it out-loud.					
22. I feel I'm making a fool of myself					
23. I believe my mind is being read.					
24. I (or others) can hear my inner thoughts outside in external space.					
25. I feel drunk					
26. Currently, events are more significant than usual.					
27. My thoughts or movements are being controlled by something or somebody else.					
28. This experience is pleasurable.					
29. I feel threatened by the researchers.					
30. My perception of my own body is altered.					

Table A1.1 The Subjective Experiences of Cannabis Questionnaire (SECQ). Items were rated on a 5-point scale 1-5 (No, Minimal, Moderate, Strong, Extreme).

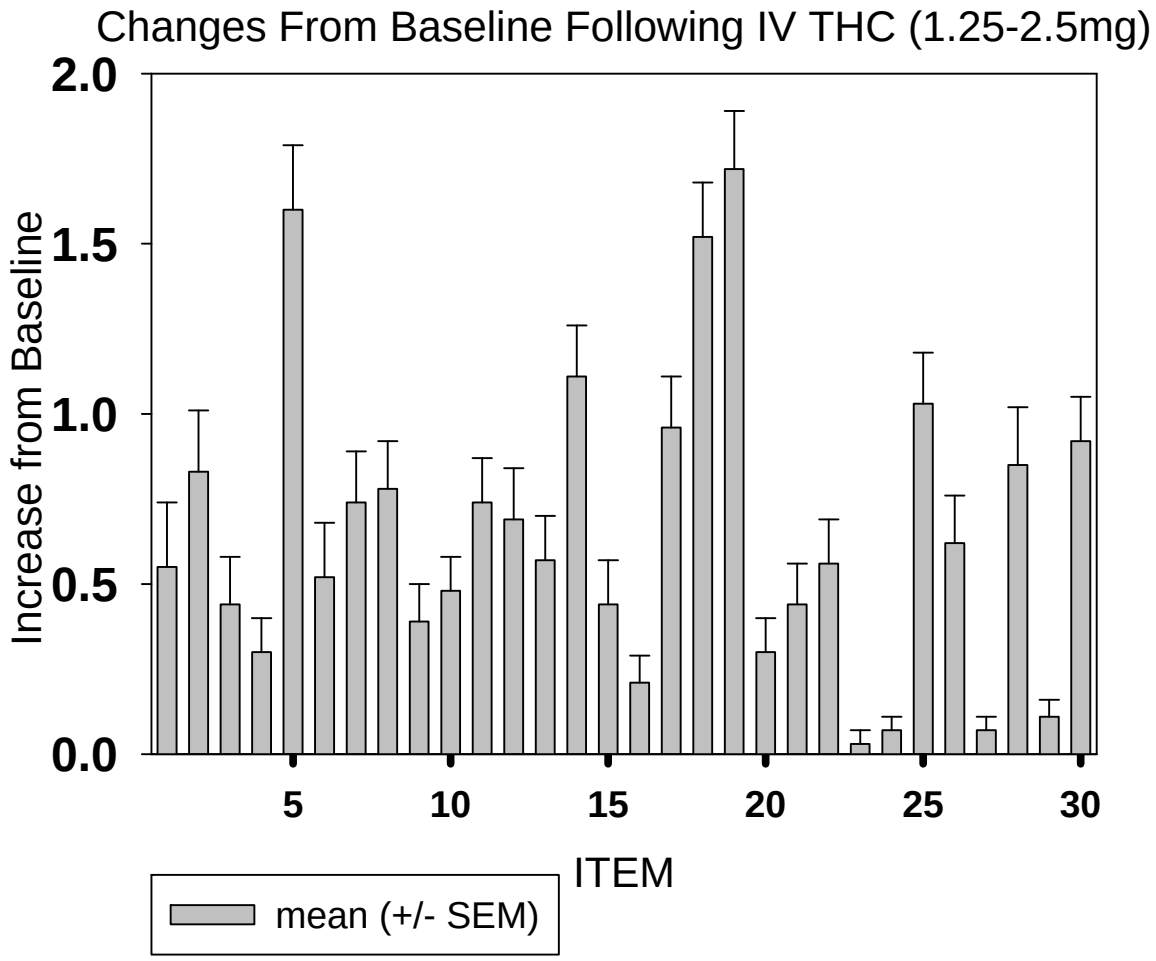


Figure A1.1 The change from baseline in SECQ scores by item, following the administration of THC (2.5mg n=3), (1.5mg n=25), (n=1.25mg n=26). The highest changes were for ITEM-5 "My perception of time has altered", ITEM-18 "I can't focus my attention" and ITEM-19 "I can't sustain my concentration".

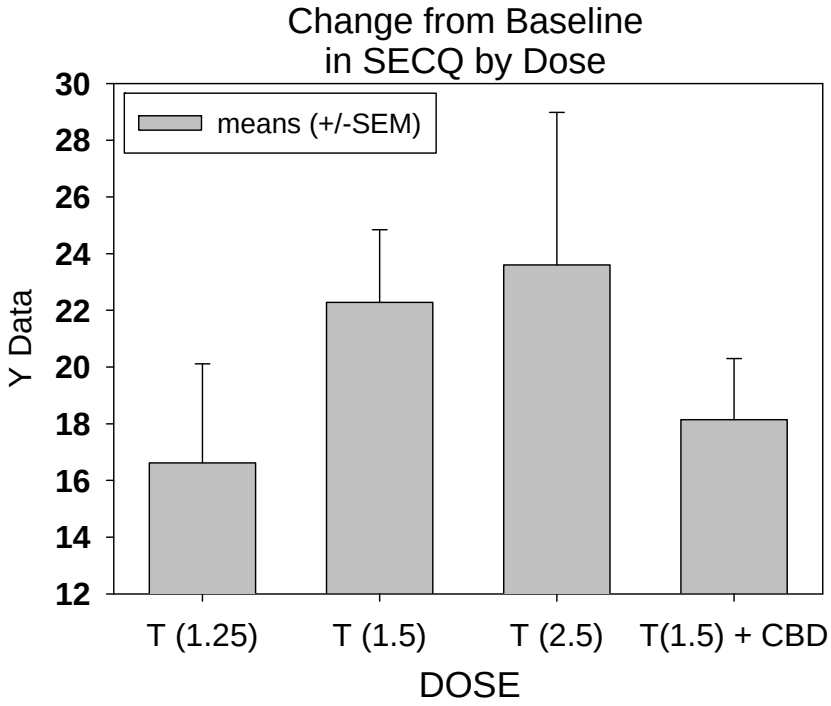


Figure A1.2a SECQ-30: Change from baseline by dose.

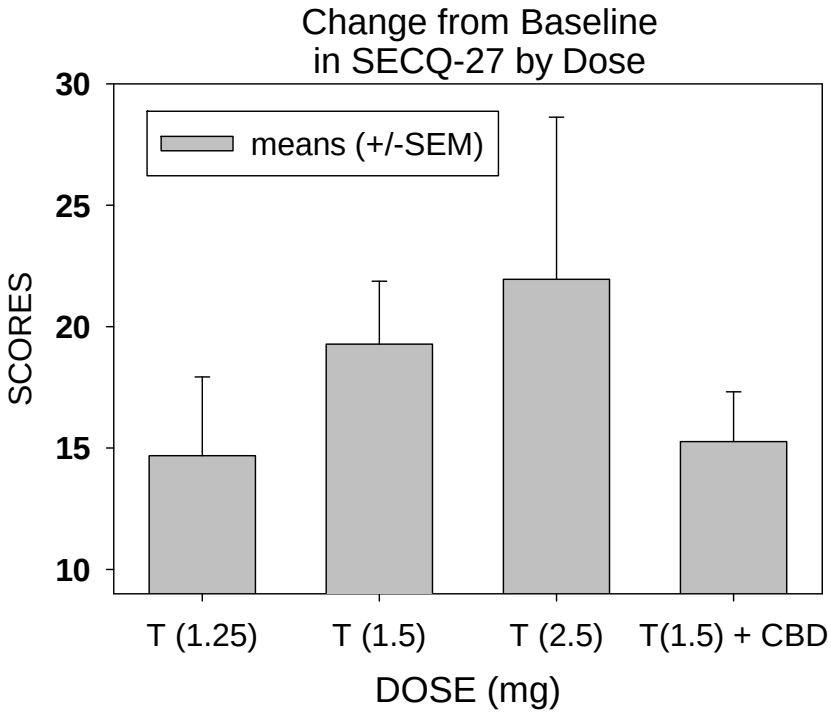


Figure A1.2b SECQ-27 (Removal of the following items: sleepy, drunk, pleasurable). Change from baseline by dose.

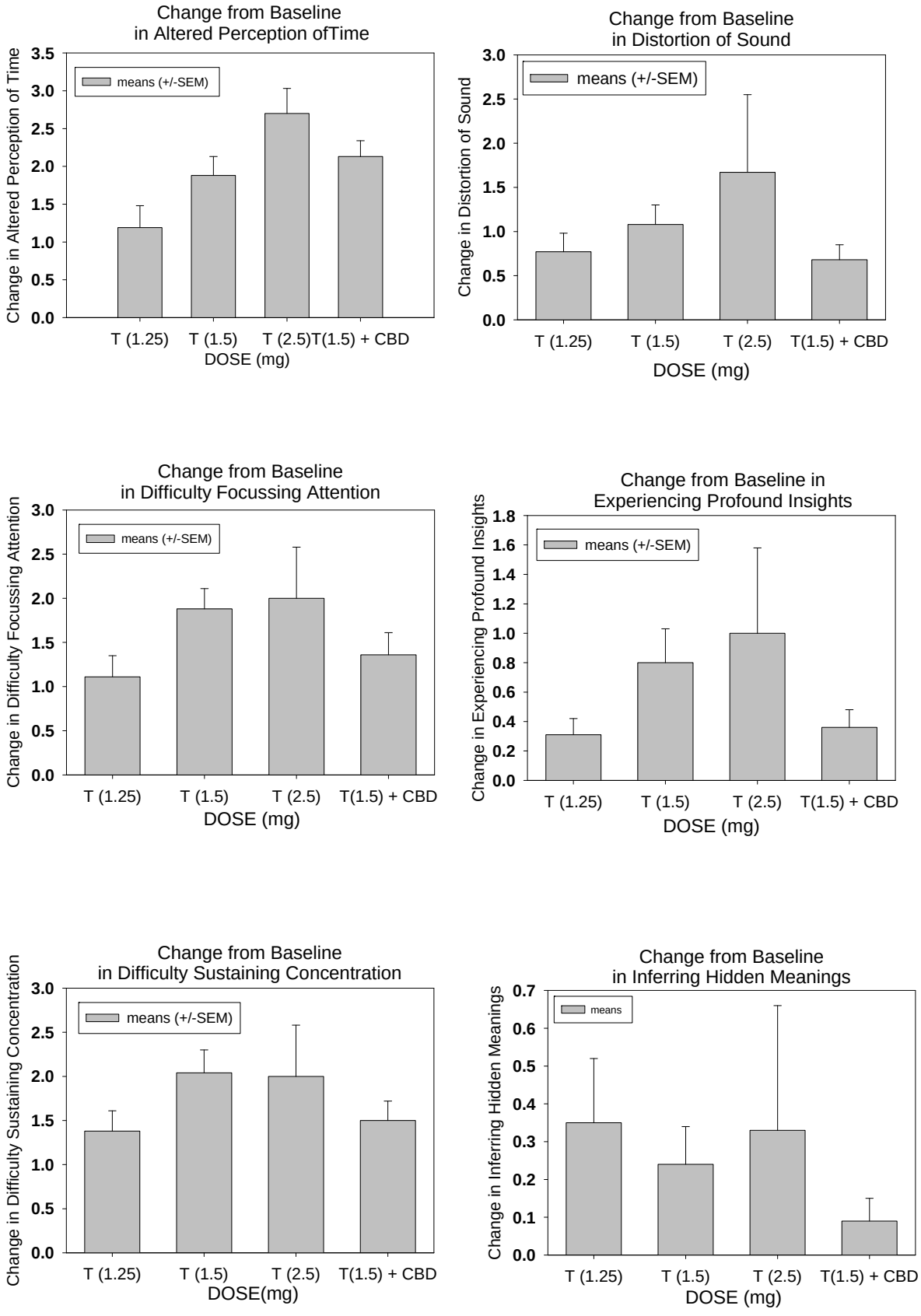


Figure A1.3 Change in selected items of the SECQ by dose

APPENDIX II: NOTES

2.1 It is important to note that experiencing psychotic symptoms, or being psychotic, is not synonymous with 'having' schizophrenia. Psychotic symptoms occur, and can be the presenting feature, in a long list of medical conditions, which include infections (HIV), endocrine disorders (pheochromocytoma), demyelinating disorders (multiple sclerosis) and so forth. Further, in contemporary psychiatry there has been a shift away from a rigidly categorical approach (mentally-well / mentally-ill) with the realization that a percentage of the population will endorse having had *schizophrenia-like* psychotic experiences - at some point in their lives, in the absence of functional disability - which resolved without treatment.

2.2 Pragmatically, in the world of the psychiatric clinic, such issues are of little relevance. Regardless of whether the most prominent psychopathology is in the domain of perception, belief or ego-boundaries, the collective term psychosis is sufficient. And our current pharmacological treatments do not draw distinctions.

Perhaps one 'solution' would be to propose the existence of a "*reality-generator*", within the CNS. This hypothesized faculty would discriminate the true from the false, and mark 'true' mental content with the "*stamp of reality*". Efficient and proper function of this '*new faculty*' would protect the mind/brain from false perceptions and false ideas, whereas failure of this '*organ*' would be the beginning of psychosis.

Of course the 'danger' of such schemes is that over time, what was clearly intended as a metaphor (or a model) becomes enshrined as actual truth. For example, it is common to encounter talk of "the default mode network", as if such a faculty actually

exists, beyond doubt – on an equal footing for instance with the faculties described by William James; attention, concentration, the will and so forth. The 'slide' into *organ*-isation is complete when the '*neural correlates*' of '*a new faculty/ mental organ*' are identified on fMRI brain scans as '*hot-spots'*.

Exploring the organic substrate/correlates of psychosis presents challenges, but these are as nothing compared to exploring that substrate within the context of schizophrenia: Negative symptoms cognitive deficits, motor symptoms, [all of which may or may not be present] fluctuations over time, the progression of some features, the diminution of others, add several layers of complexity. The 'Group of Schizophrenias' emerged from German academic psychiatry, bringing some order to the nosological chaos which had been present since [at least] the time of Griesinger (1817-1868), but 'group' seems to have been relegated to some degree, because it is commonplace to hear schizophrenia referred to as if it were a single, well-demarcated disease (akin to Huntington's for example).

2.3 This is a statement of the 'natural attitude', certainly for neuroscientists, and perhaps even for the majority of psychologists, but as recently as 1994 someone of the stature of Francis Crick was required to 'legitimize' the idea that brain operations == mind operations, in (surely with tongue-in-cheek) "*The astonishing Hypothesis*" Simon & Schuster, London.

2.4 There are two major theoretical accounts of how neural tissue "performs its computations".

The first account postulates the existence of 'special cells' at the top of a processing hierarchy. These cells are less 'concerned' by the raw 'building blocks' of sensory experience orientation, brightness, colour, pitch etc. Instead, they respond ('fire') to whole objects (Gestalts), regardless of perspective, illumination and all the other idiosyncrasies that make up a perceptual scene. The metaphor of the 'grandmother cell' captures the idea[209]. "Each time my grandmother comes into consciousness, via any of the sensory channels or in imagination, a 'special' cell, somewhere in my brain, is active". The main criticism of the 'grandmother cell' hypothesis is that there are far more potential percepts, than available neurons. Another criticism is that by focusing exclusively on feed-forward pathways, the hypothesis ignores the anatomical 'reality' of extensive feedback pathways. Nevertheless, in-vivo *electrophysiological* work in humans undergoing neurosurgical procedures has provided evidence that there are neurons in the medial temporal lobe, which have the characteristics of grandmother cells[210].

The second account prioritizes flexible, dynamic assemblies of neurons over 'special' cells. An assembly is defined as a constellation of neurons, which are firing action-potentials within the same narrow time-window (synchronously). Here, processing is a more 'democratic affair', and no special cells are required. Feedback and feed-forward connections are equally important, and the network (the assembly) reaches a consensus. Assemblies are transient entities, emerging for a period before 'dissolving', perhaps to 'reappear' at a later instant. A temporarily 'dominant' assembly may '*recruit*' other 'partners'. Allegiances are flexible, with co-operation at one instant and competition at another. And over longer periods of time, assemblies can become – *stronger*; by virtue of sheer repetition and the 'rules' of long-term-

potentiation (LTP), particularly if monoamine systems are co-active - or *weaker*; if the 'content' is fleeting or insignificant. Oscillations (rhythms) provide a timing device, to ensure that the right cells fire at the right time. The individual cells within the network give rise to the rhythm, but are themselves constrained by the rhythm[119]. Synchronized gamma (30–200 Hz) rhythms 'bind' local assemblies, whereas lower frequencies (theta, alpha, and beta) sub-serve long-distance communication between brain areas[211-213].

2.5 One effect of the interest in rhythms/oscillations was the 'resurrection' of moribund technology, specifically EEG. The 'sister' technology MEG magnetoencephalography, was (until recently) virtually unheard of outside Finland, Germany and the USA. MEG is new and expensive but offers little advantage over EEG aside from less 'spatial smearing' of the magnetic compared to the electrical signal. MEG also has issues around the head having to remain still etc. For both technologies, the 'inverse problem' applies: Of the potentially infinite number of solutions that account for measurements made at the scalp, which one is correct?

The temporal resolution of EEG/MEG far surpasses that from any other 'neuroimaging' modality. Recording at >1000Hz is routine, meaning that EEG/MEG can resolve events in the same timescale within which neuronal tissue operates (i.e. the millisecond range).

The temporal resolution of functional MRI imaging approaches 1-2 seconds. Although the novelty-value has long faded, fMRI still produces the most eye-catching

'pictures', if PET is not included in the list. Leaving aside the debate about whether BOLD fMRI represents vascular responses or neural-activation, the more troubling issue, the philosophical issue, is that the mind seems to be just too fast to be 'caught' on an fMRI camera.

There is another philosophical issue, which is better known. This is the phrenology charge. Because the BOLD technique involves subtraction of scans under one condition from scans under another condition, it always produces a localised signal, perhaps a hot spot here or a cold spot there [or in the hands of the un-checked beginner, a multitude of hot and cold spots]. It is a '*phrenological*' method from the outset, so it is not at all surprising to obtain '*phrenological*' solutions.

Of course it would be absurd to argue that one technology is better than another. This would be like (*amateur*) astronomers arguing over whether an X-ray telescope is better than a radio telescope for viewing the heavens.

2.6 The story started with oscillations and the precise temporal organisation of spikes (action-potentials), but now encompasses 'phase precession' and other exotic phenomena such as spike-timing-dependent-plasticity (STDP).

Spike-timing-dependent plasticity (STDP) depends on the conjunction of pre and post-synaptic events, within a narrow time envelope, of the order of *tens of milliseconds* or so. In the most straightforward version, a synapse is strengthened if a pre-synaptic input occurs *immediately prior* to a post-synaptic action potential (AP). If on the other hand, the input arrives *in the immediate aftermath* of a post-synaptic

AP, the synapse is weakened. Pre and post-synaptic events beyond the critical time-window (i.e. unpaired 'events') leave synaptic strength unchanged[214].

Four aspects of STDP are notable[124]:

- Unlike '*conventional plasticity*, STDP can be evoked without the need for high intensity pre-synaptic 'blasts' (at 50-100Hz), which rarely occur in 'normal' physiology. Instead one or two pre and post-synaptic spikes suffice. Timing is the critical variable. Energy requirements are kept low.

- Conventional neuromodulators appear to 'tweak' STDP. Actually 'tweak' is an understatement. The presence of a modulator such as dopamine can transform a normal pre-> post *strengthening* into a *depression* instead. More succinctly, dopamine can determine the *direction* of plasticity (+ or -).

- The critical time window of STDP (tens of milliseconds) is in exactly the same 'ballpark' as network oscillations in the gamma band (period ~25ms).

- Endocannabinoids mediate spike-timing dependent LTD.

2.7 Part of the difficulty is that we are accustomed to talking about the brain as if its operations can be described by linear cause-effect relationships. This is common in cognitive psychology, but also in 'biological' psychiatry. (Think of the ubiquitous box and arrow diagram). The term 'complex' usually appears at some point, (not to signify that things are difficult to understand), but in acknowledgement that a linear '*geometry*' is insufficient to capture the operations of the CNS.

2.8 It is unclear why stimulant psychosis requires repeated dosing rather than a single exposure to the drug[215, 216]. At least in healthy participants, the acute effects of stimulants are quite unlike paranoid psychoses. According to Freud, "*Cocaine brings about an exhilaration and lasting euphoria which in no way differs from the normal euphoria of the healthy person…You perceive an increase of self-control and possess more vitality and capacity for work…In other words you are simply normal, and it is soon hard to believe that you are under the influence of any drug*. Jones E *The life and work of Sigmund Freud* Basic Books: New-York 1953."

Since repeated dosing appears to be a pre-requisite, an adaptive CNS process may be involved in the neuropsychiatric sequelae of stimulants[117, 217-219].

One of the outcomes of repeated stimulant administration is a functional up-regulation of striatal CB_1 receptors[220]. Furthermore, several recent studies have shown that either CB_1 knockout or blockade of CB_1 receptors by potent CB1 antagonists impairs stimulant sensitization[221-223]. In a particularly elegant design it was demonstrated that microinjections of a potent CB_1 antagonist directly into the ventral striatum reduced the expression of behavioral sensitization to methamphetamine[117]. Findings with the first generation CB_1 blocker rimonabant in sensitization paradigms have been inconsistent[223, 224], but rimonabant, as opposed to newer CB_1 antagonists, only partially blocks the effects of THC in humans[126, 225] (Huestis et al., 2001; Zuurman et al., 2010) which might explain why an early trial of rimonabant in schizophrenia failed [226].

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